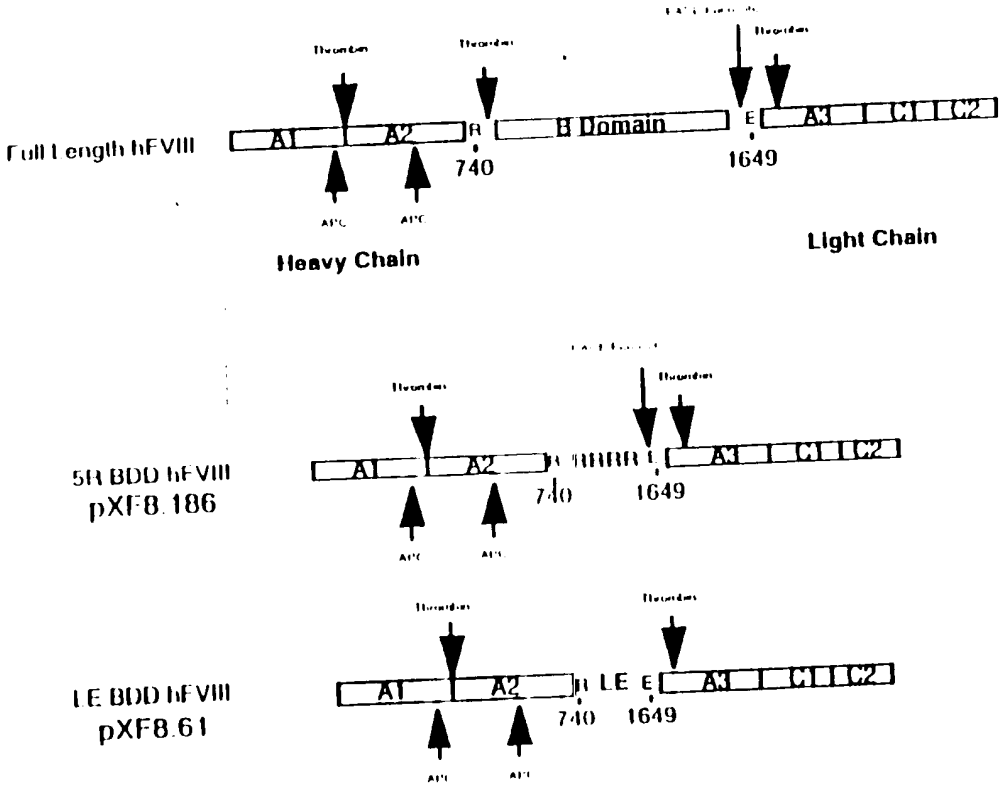
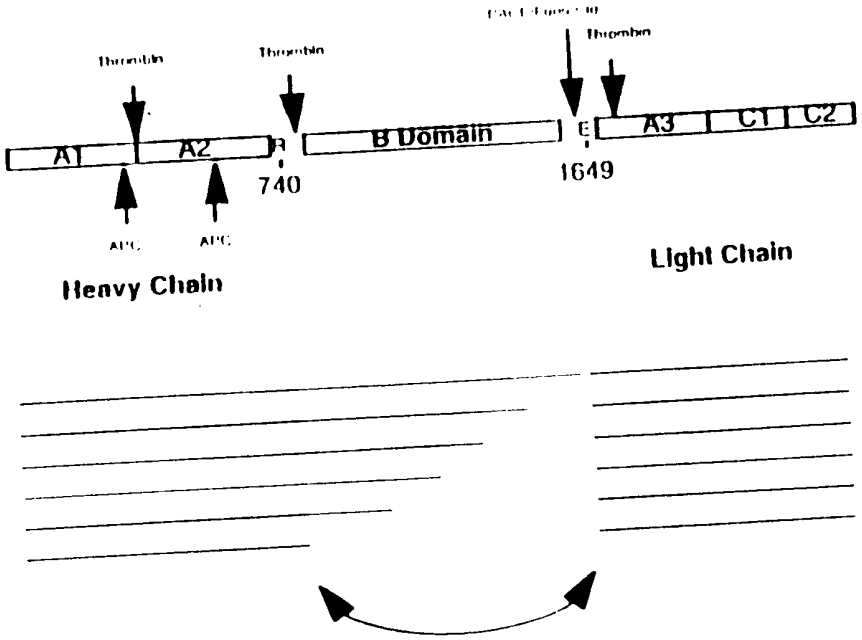
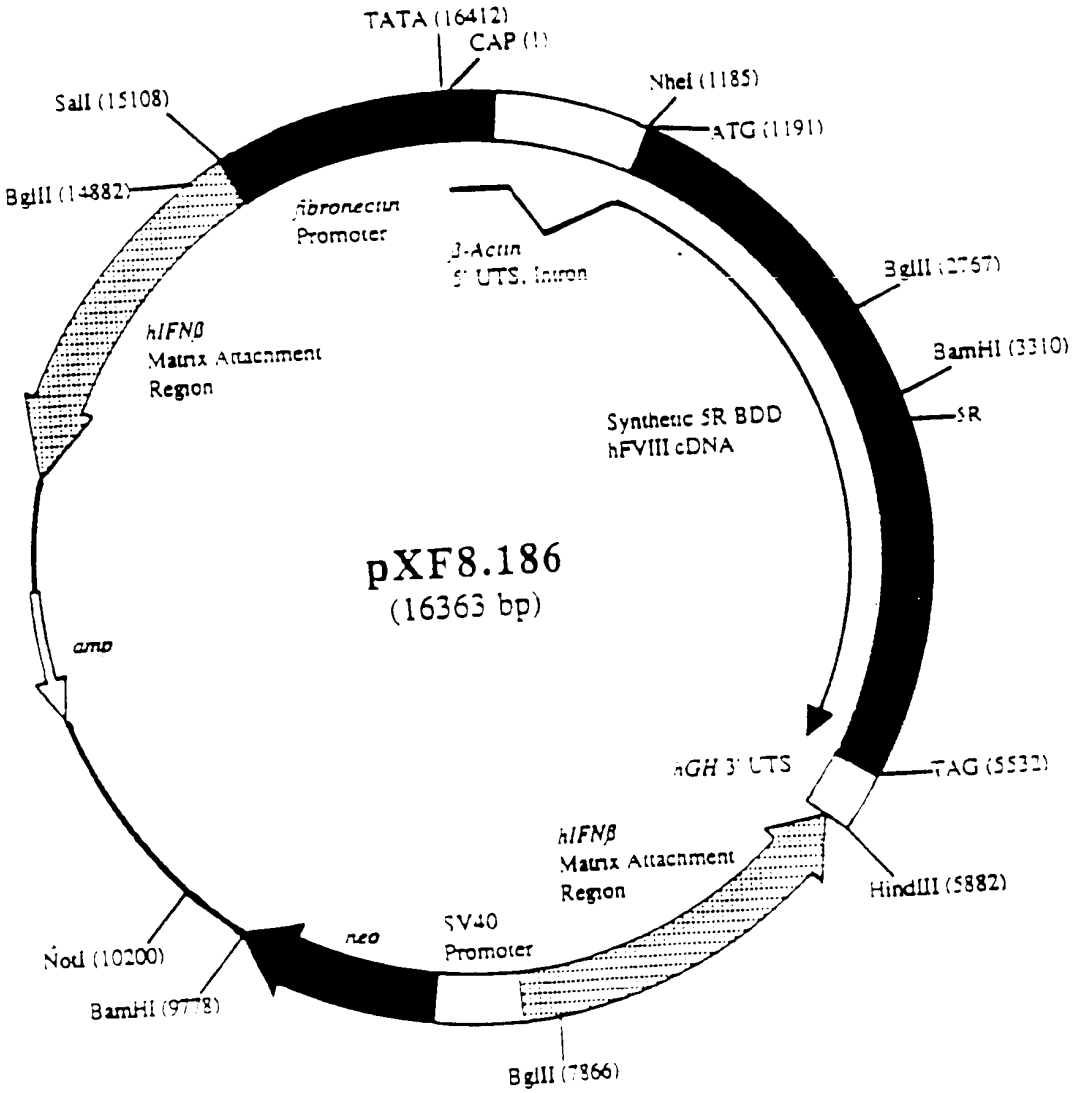

Full Length hFVIII
A1
A2
R
740
B Domain
E
1649
A3
C1
C2
Heavy Chain
Light Chain
5R BDD hFVIII
pXF8.186
LE BDD hFVIII
pXF8.61
LE

FIG. 1



Heterogeneity of hFVIII is due to proteolysis within the B-domain

FIG. 2


TATA (16412)
CAP (1)
NheI (1185)
ATG (1191)
SalI (15108)
BglII (14882)
fibronectin
Promoter
β-Actin
5' UTS, Intron
BglII (2767)
BamHI (3310)
SR
hIFNβ
Matrix Attachment
Region
Synthetic SR BDD
hFVIII cDNA
pXF8.186
(16363 bp)
amp
hGH 3' UTS
TAG (5532)
hIFNβ
Matrix Attachment
Region
HindIII (5882)
NotI (10200)
neo
SV40
Promoter
BamHI (9778)
BglII (7866)

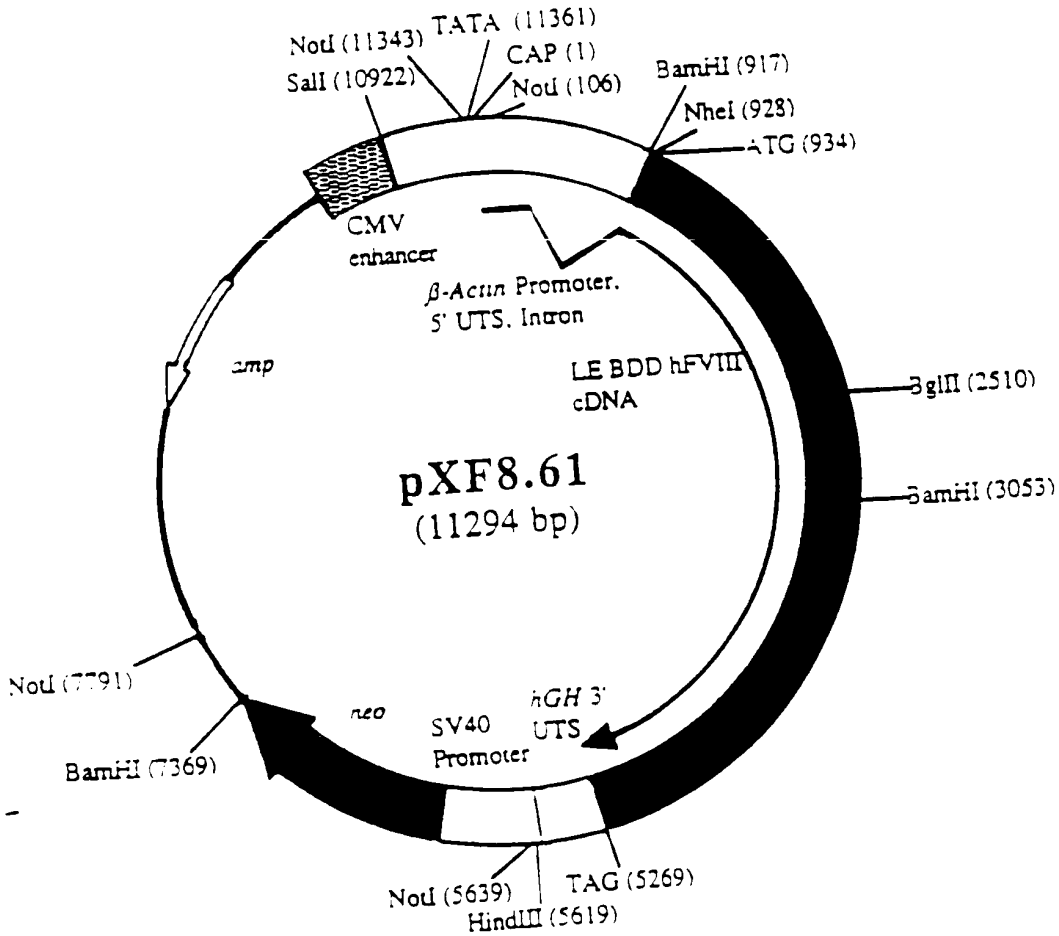

NotI (11343)
TATA (11361)
CAP (1)
NotI (106)
SalI (10922)
BamHI (917)
NheI (928)
ATG (934)
CMV
enhancer
β-Actin Promoter.
5' UTS. Intron
amp
LE BDD hFVIII
cDNA
BglII (2510)
BamHI (3053)
pXF8.61
(11294 bp)
NotI (7791)
BamHI (7369)
neo
SV40
Promoter
hGH 3'
UTS
NotI (5639)
HindIII (5619)
TAG (5269)

FIG. 4

Fragment A

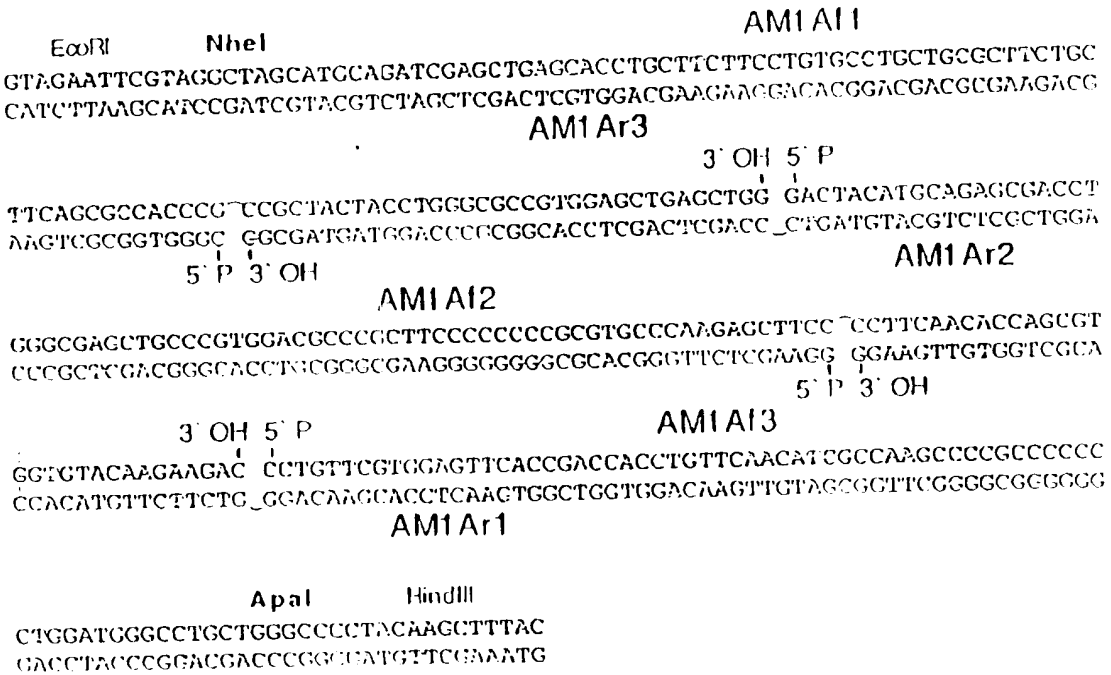


FIG. 5 (1 of 14)

Fragment B

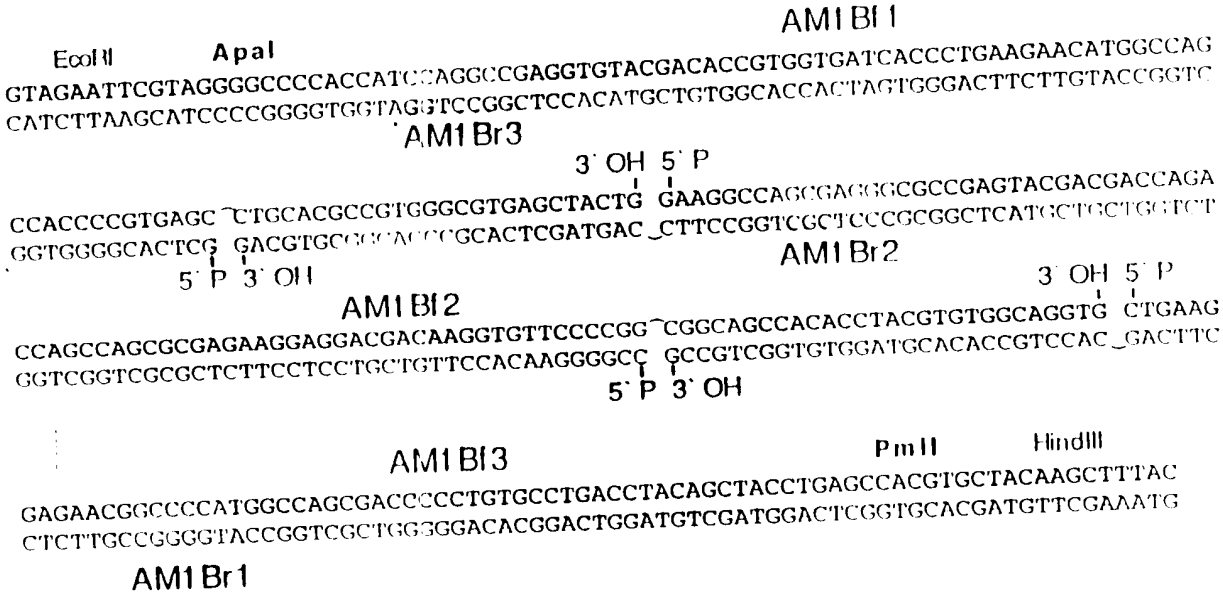


FIG. 5 (2 of 14)

Fragment C

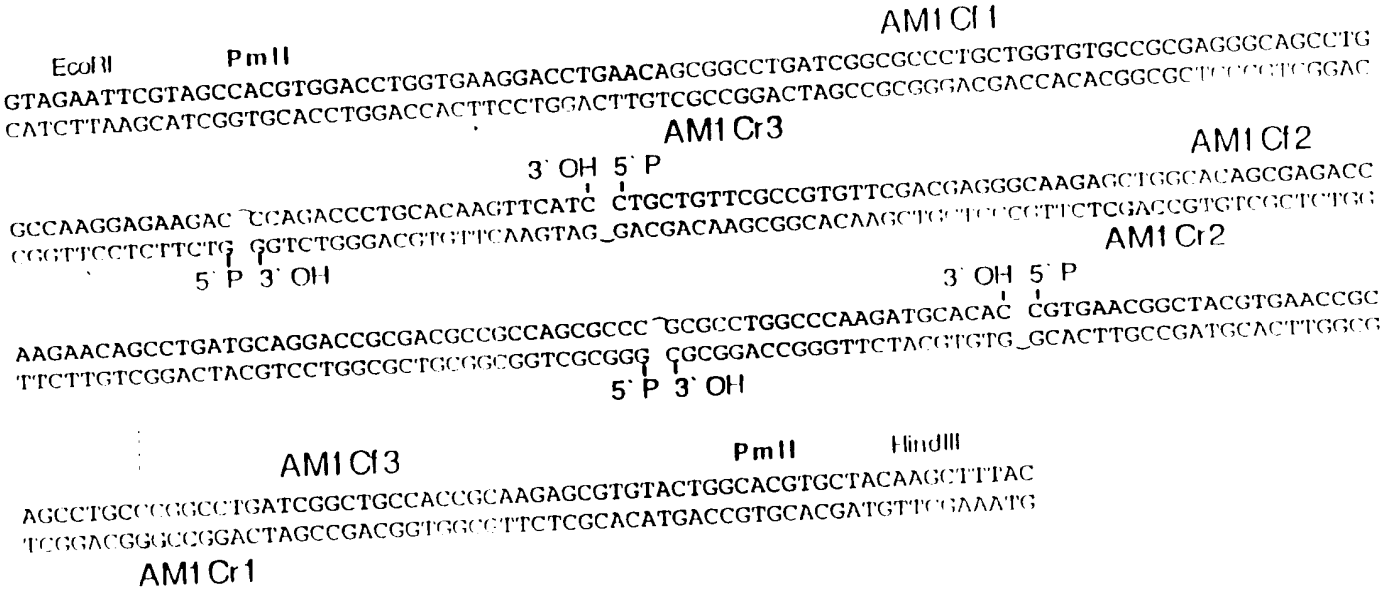


FIG. 5 (3 of 14)

Fragment D

FIG. 5 (4 of 14)

Fragment E

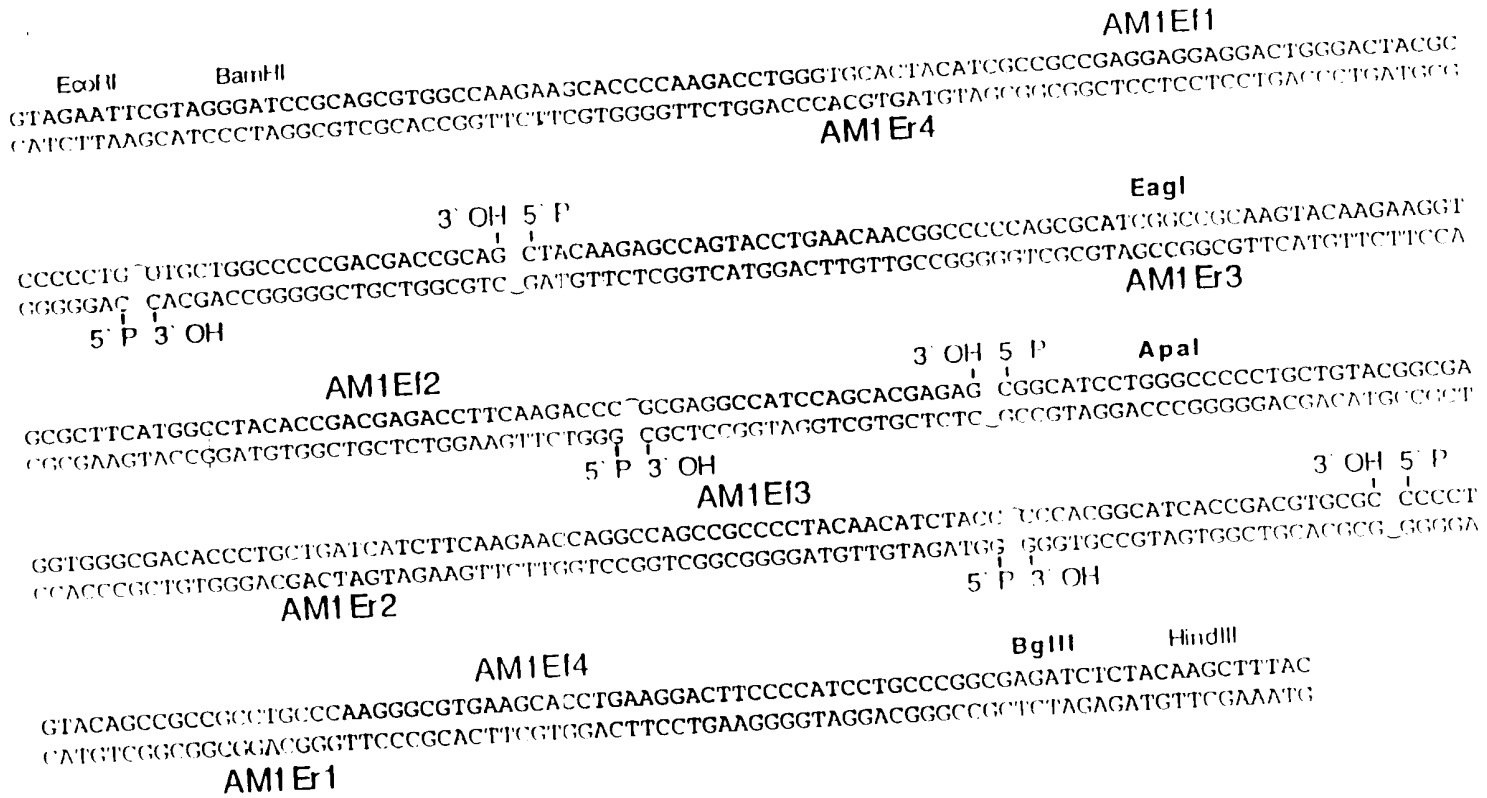


FIG. 5 (5 of 14)

Fragment F

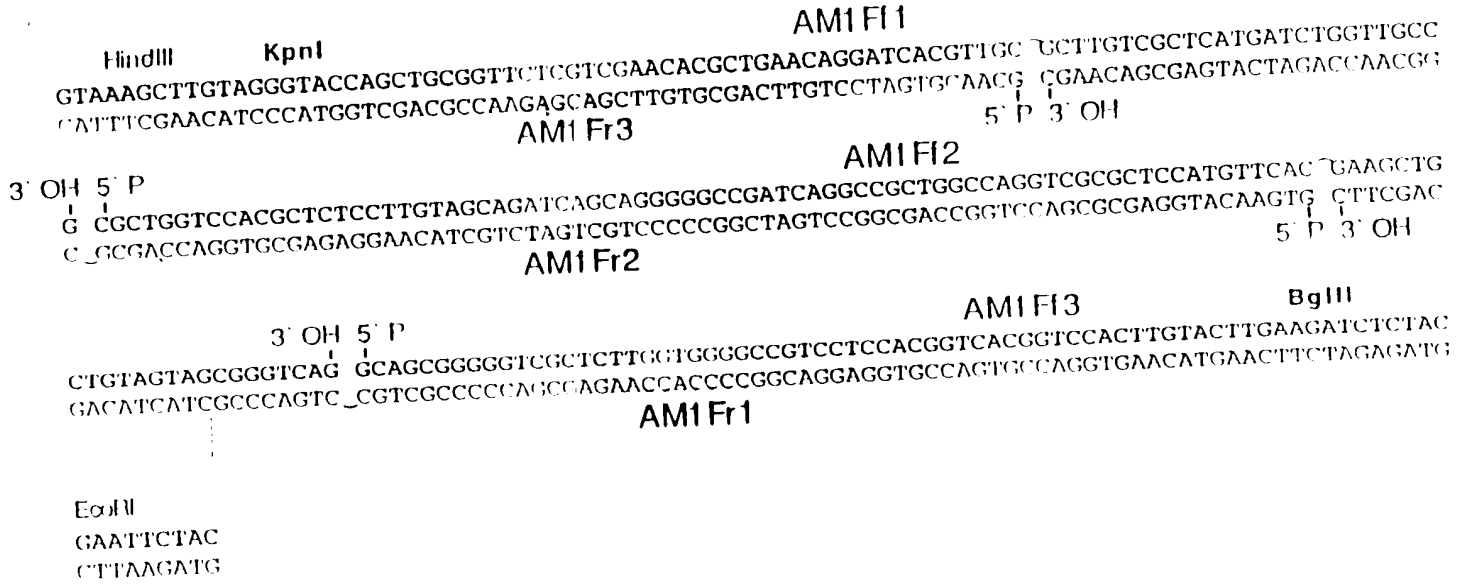


FIG. 5 (6 of 14)

Fragment G

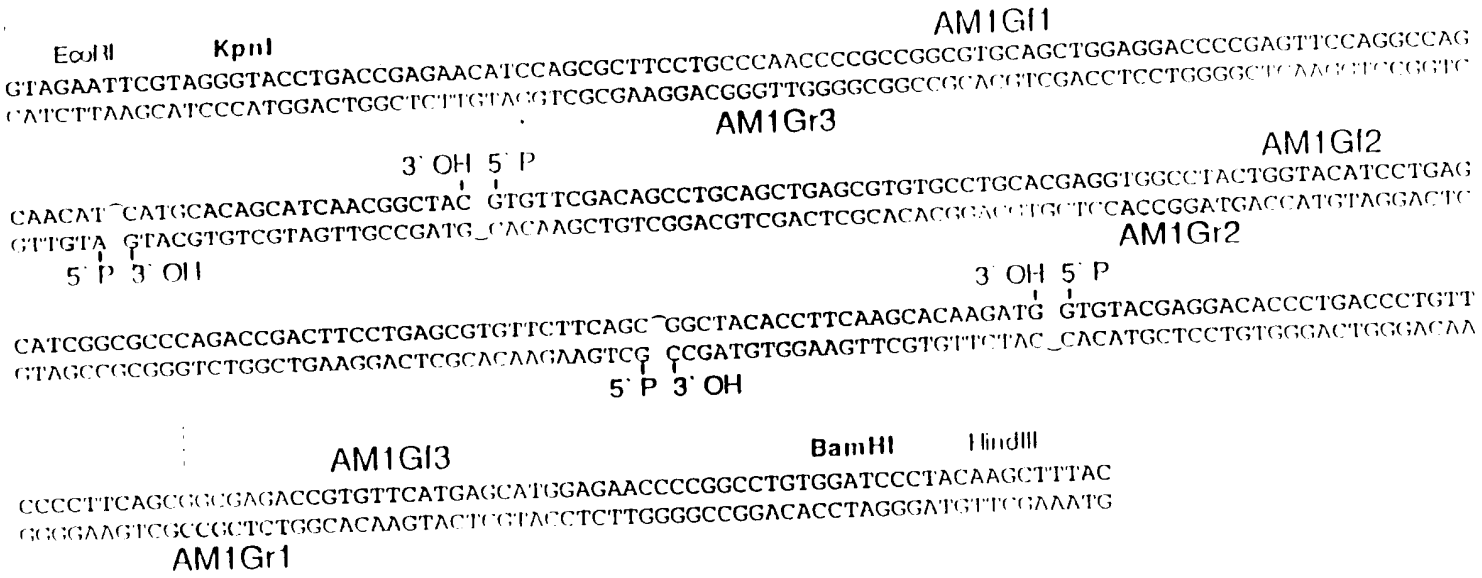


FIG. 5 (7 of 14)

Fragment H

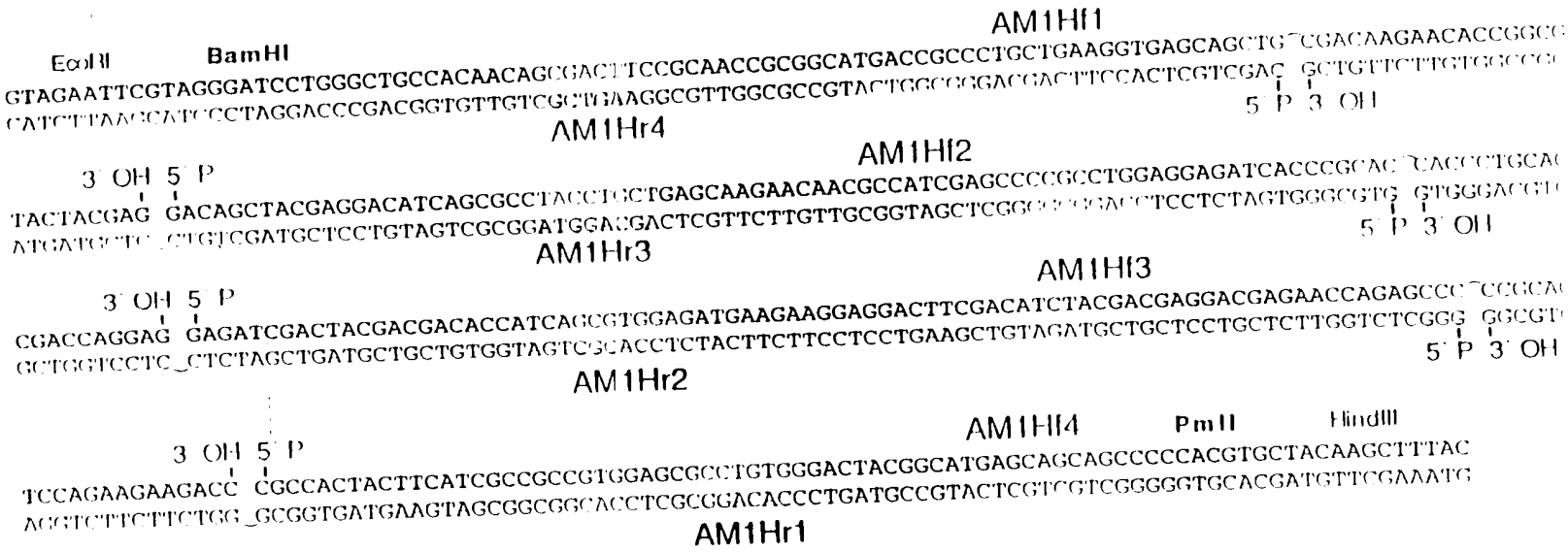


FIG. 5 (8 of 14)

Fragment I

FIG. 5 (9 of 14)

Fragment J

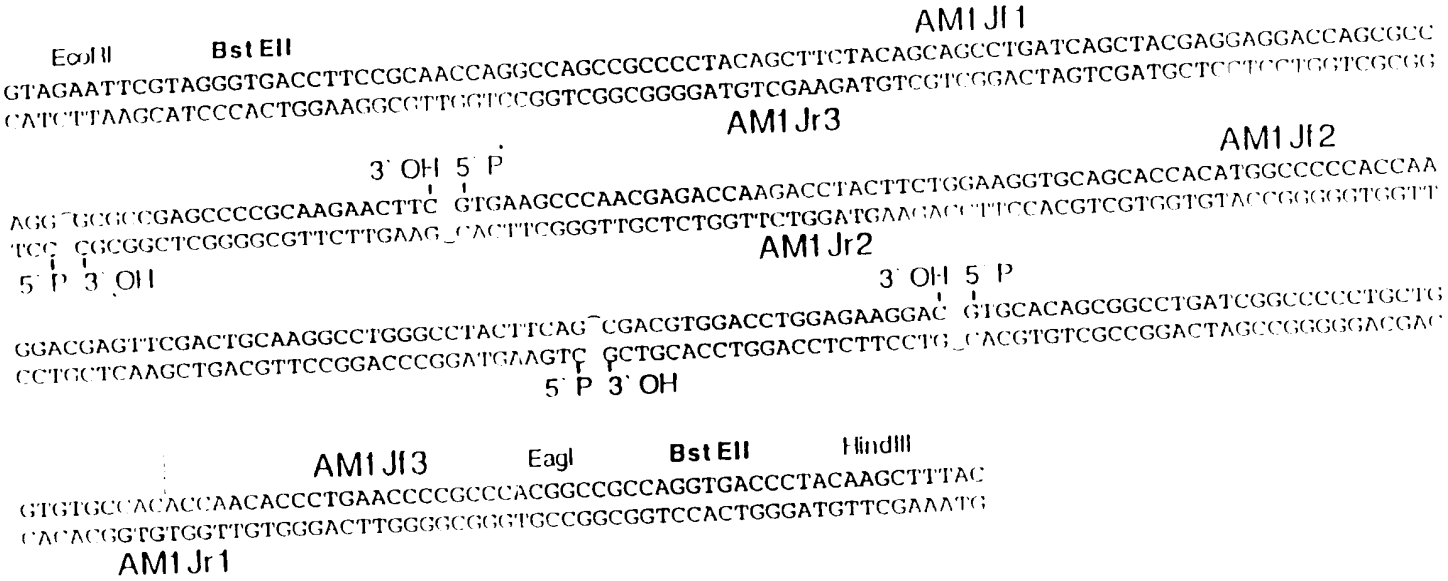


FIG. 5 (10 of 14)

Fragment K

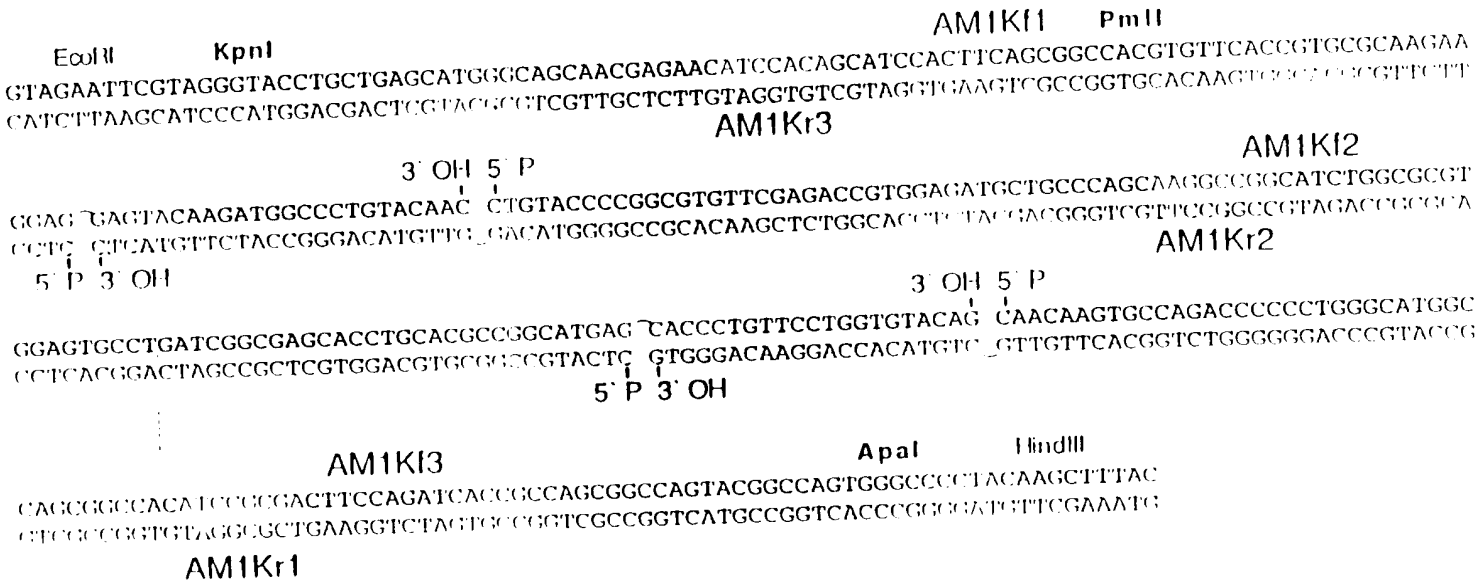


FIG. 5 (11 of 14)

Fragment L

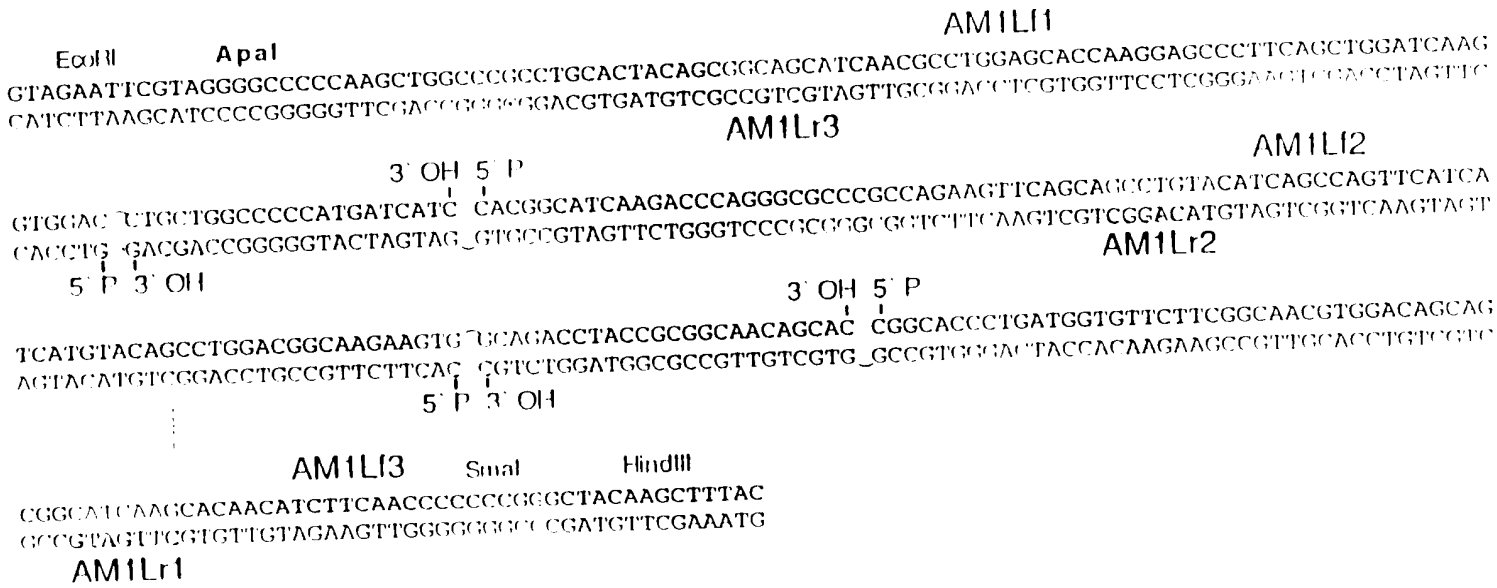


FIG. 5 (12 of 14)

Fragment M

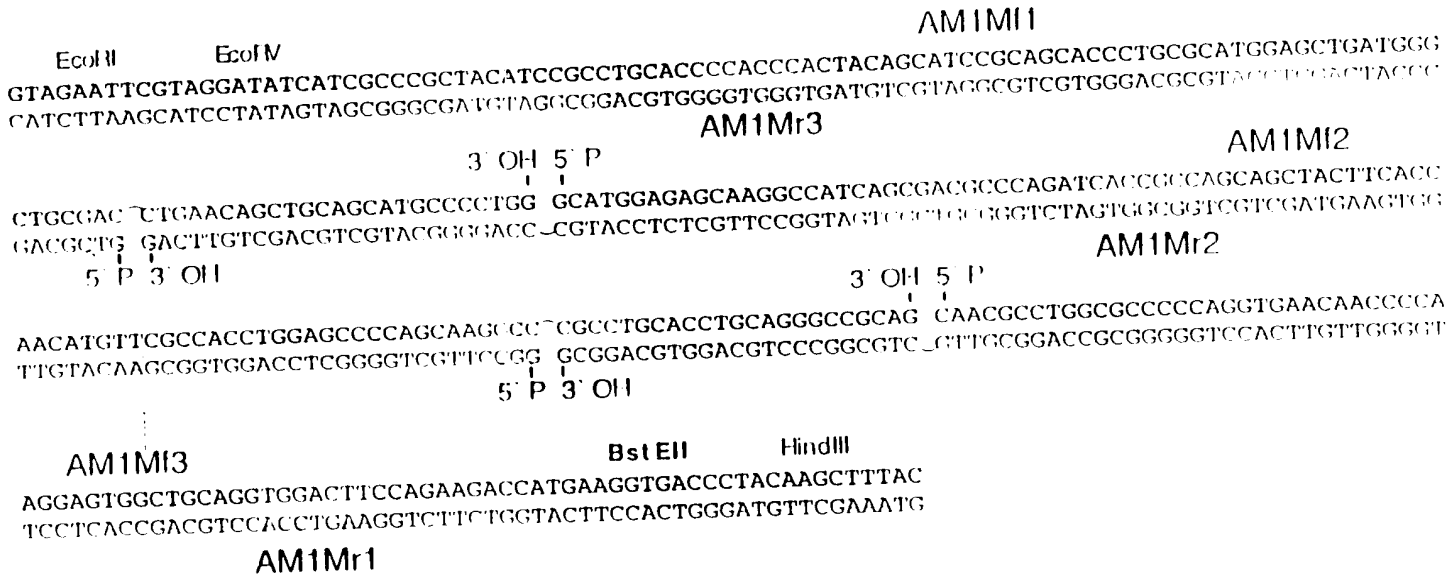


FIG. 5 (13 of 14)

Fragment N

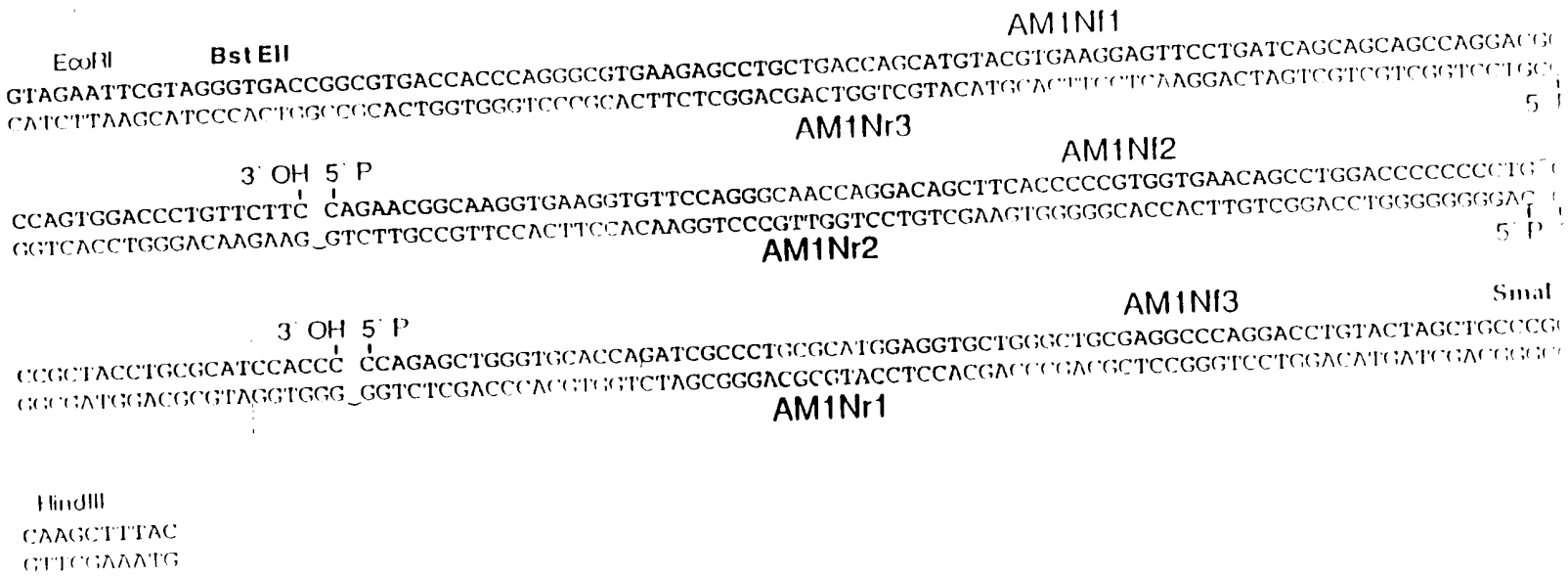


FIG. 5 (14 of 14)

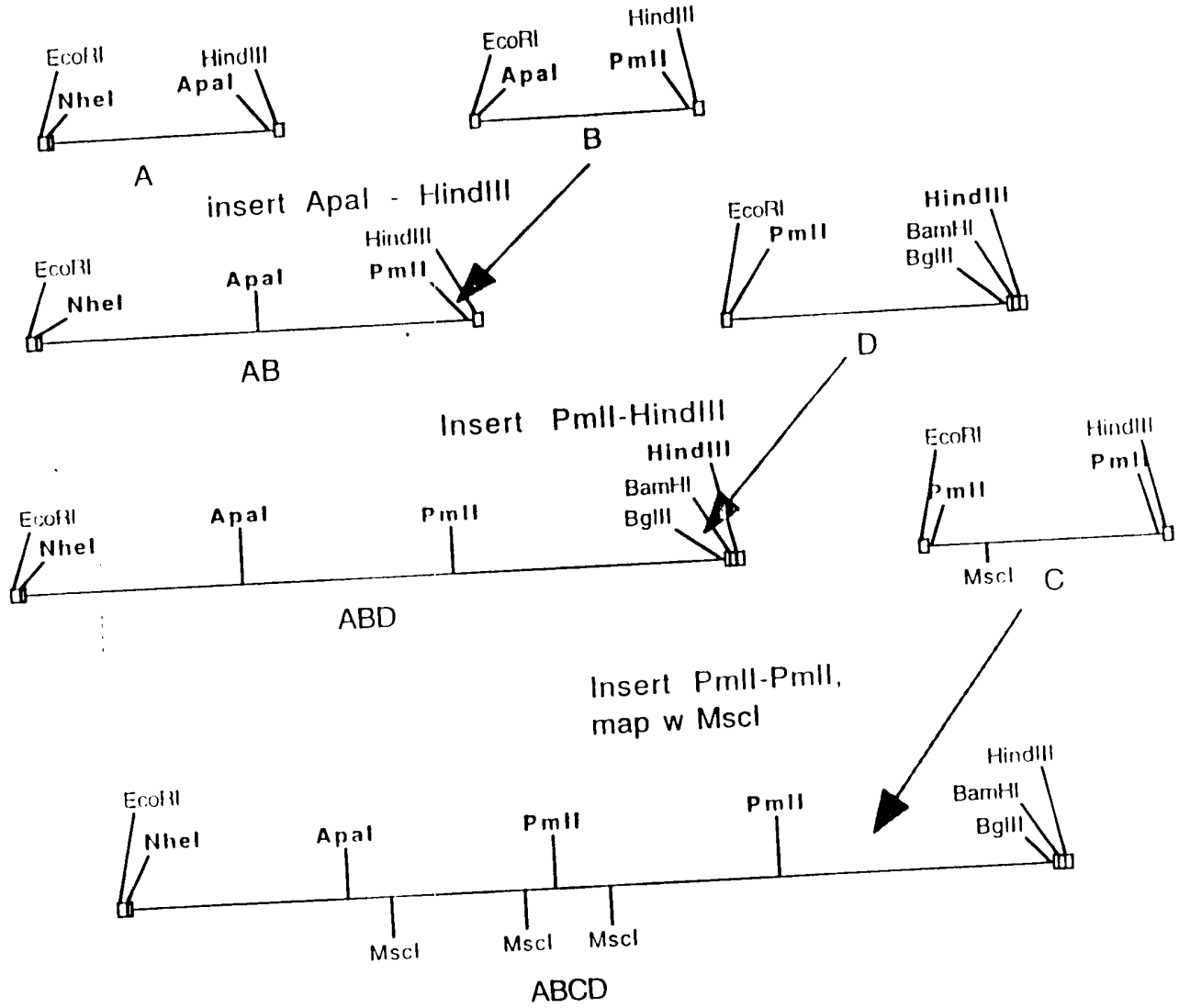

EcoRI
HindIII
NheI
ApaI
A
EcoRI
HindIII
ApaI
PmlI
B
insert ApaI - HindIII
EcoRI
NheI
ApaI
HindIII
PmlI
AB
EcoRI
PmlI
HindIII
BamHI
BglII
D
Insert PmlI-HindIII
EcoRI
NheI
ApaI
PmlI
HindIII
BamHI
BglII
ABD
EcoRI
PmlI
HindIII
PmlI
MscI
C
Insert PmlI-PmlI,
map w MscI
EcoRI
NheI
ApaI
PmlI
PmlI
HindIII
BamHI
BglII
MscI
MscI
MscI
ABCD

FIG. 6 (1 of 5)

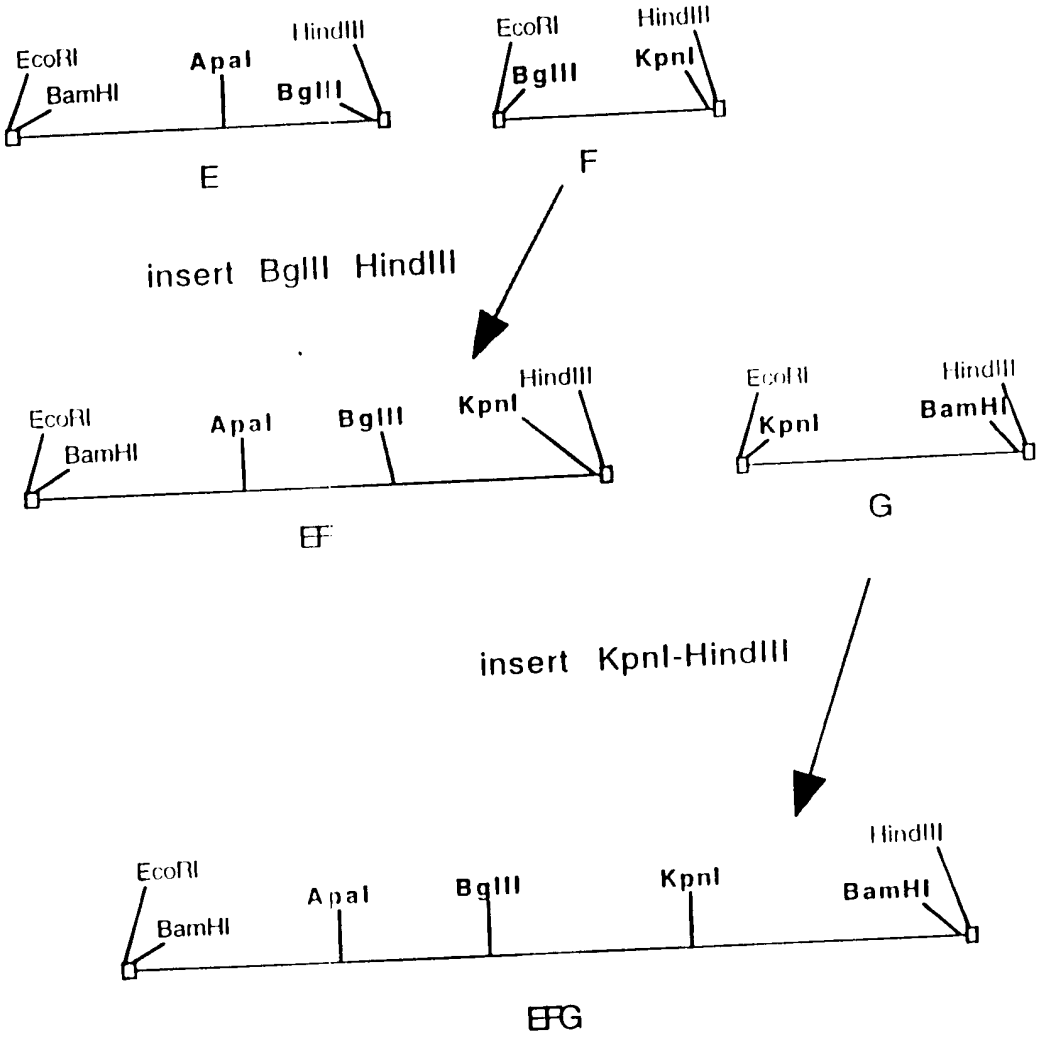

EcoRI
BamHI
ApaI
HindIII
BglII
E
EcoRI
BglII
HindIII
KpnI
F
insert BglII HindIII
EcoRI
BamHI
ApaI
BglII
KpnI
HindIII
EF
EcoRI
KpnI
HindIII
BamHI
G
insert KpnI-HindIII
EcoRI
BamHI
ApaI
BglII
KpnI
HindIII
BamHI
EFG

FIG. 6 (2 of 5)

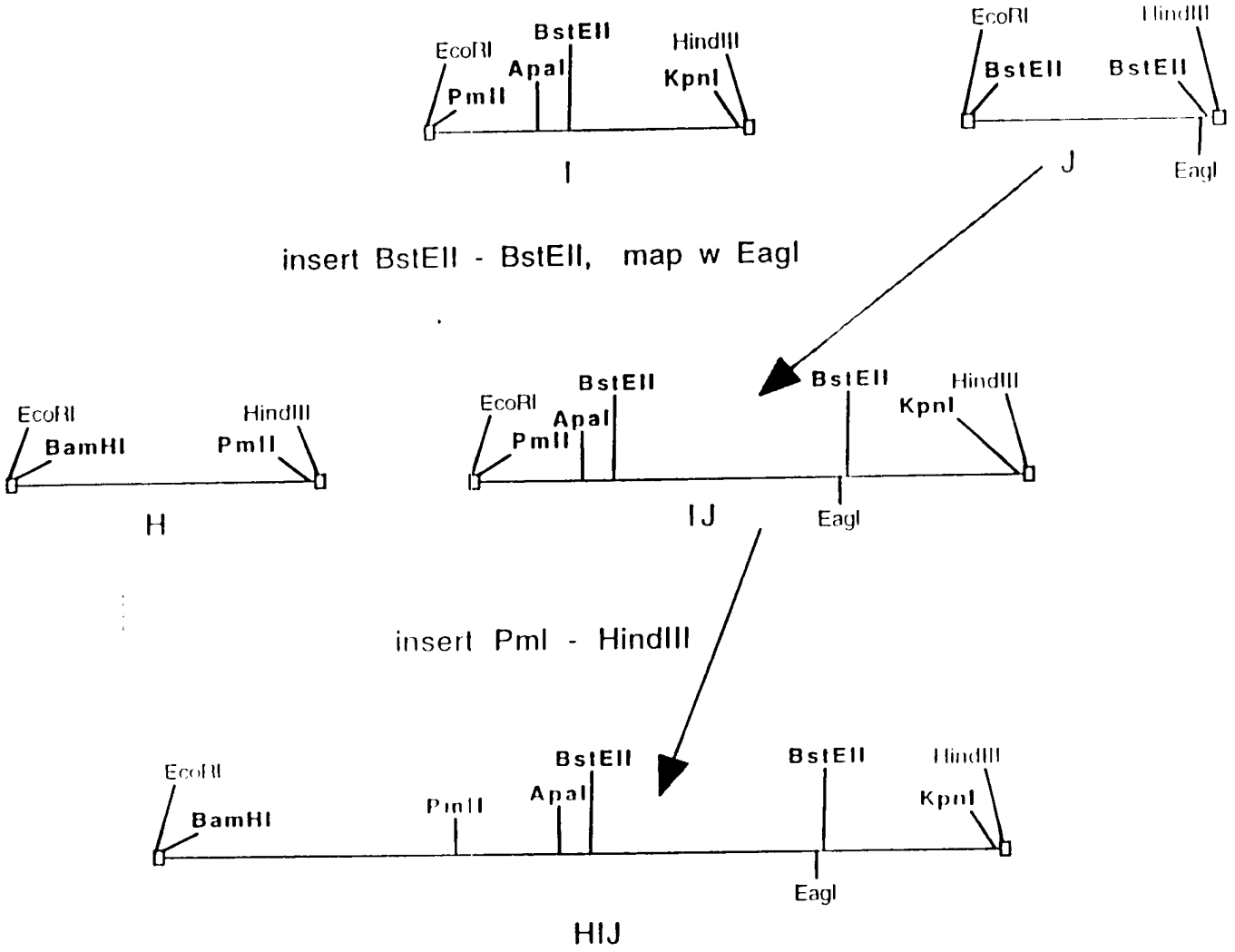

EcoRI
BstEII
ApaI
PmlI
HindIII
KpnI
I
EcoRI
HindIII
BstEII
BstEII
J
EagI
insert BstEII - BstEII, map w EagI
EcoRI
BamHI
PmlI
HindIII
H
EcoRI
BstEII
ApaI
PmlI
BstEII
KpnI
HindIII
IJ
EagI
insert PmI - HindIII
EcoRI
BamHI
PmlI
BstEII
ApaI
BstEII
KpnI
HindIII
EagI
HIJ

FIG. 6 (3 of 5)

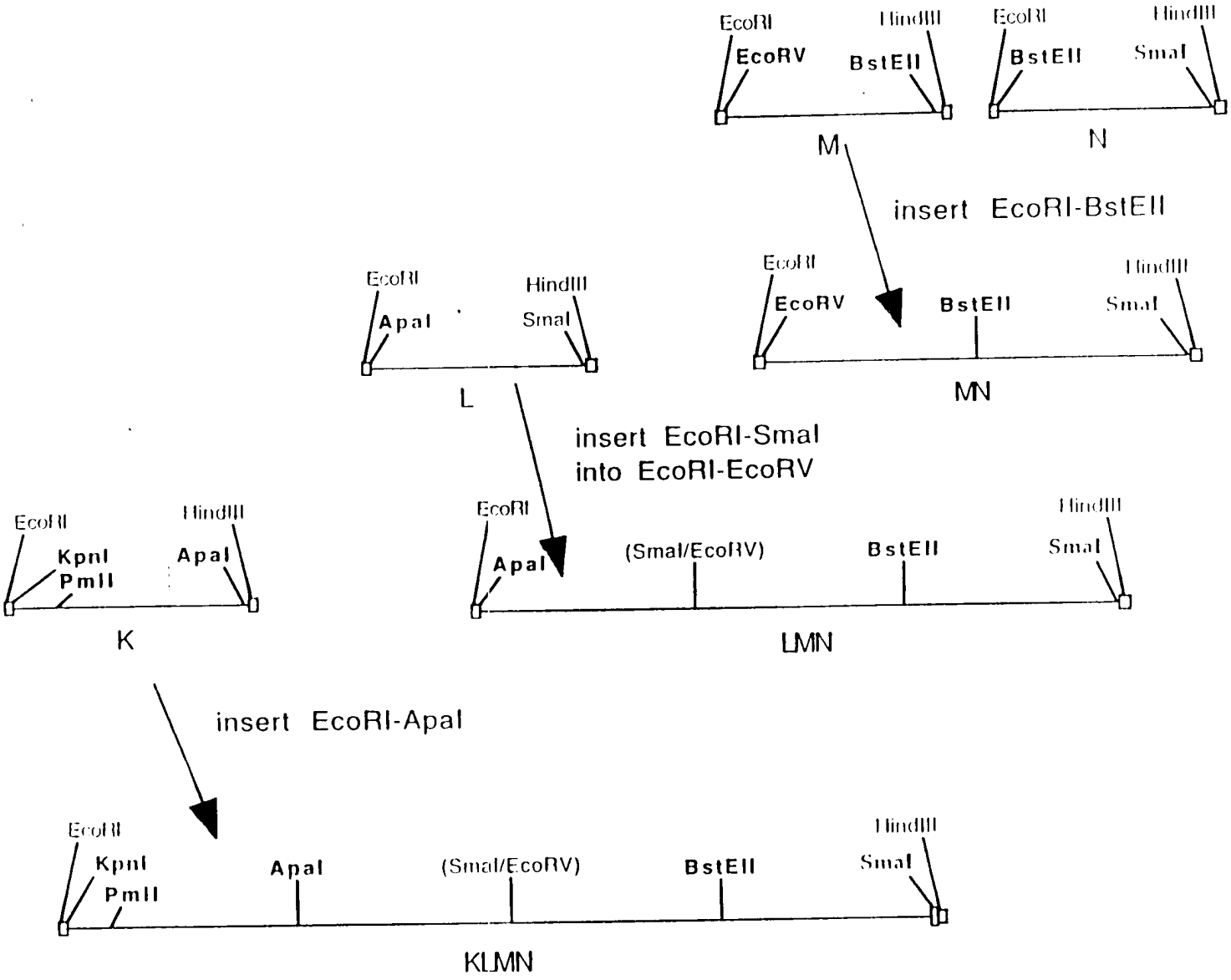

EcoRI
HindIII
EcoRV
BstEII
M
EcoRI
HindIII
BstEII
SmaI
N
insert EcoRI-BstEII
EcoRI
HindIII
EcoRV
BstEII
SmaI
MN
EcoRI
HindIII
ApaI
SmaI
L
insert EcoRI-SmaI
into EcoRI-EcoRV
EcoRI
HindIII
KpnI
PmlI
ApaI
K
EcoRI
HindIII
ApaI
(SmaI/EcoRV)
BstEII
SmaI
LMN
insert EcoRI-ApaI
EcoRI
HindIII
KpnI
PmlI
ApaI
(SmaI/EcoRV)
BstEII
SmaI
KLMN

FIG. 6 (4 of 5)

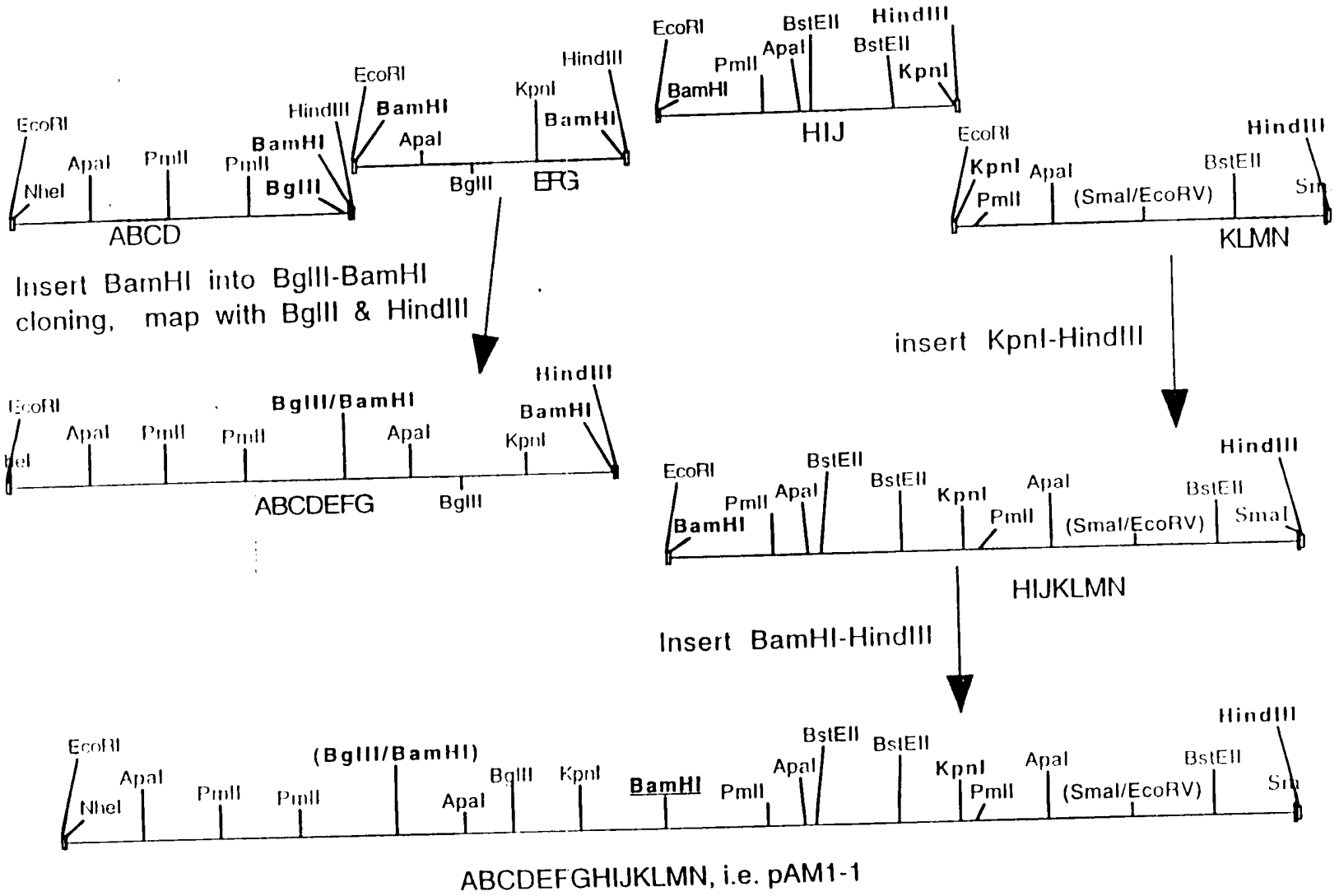
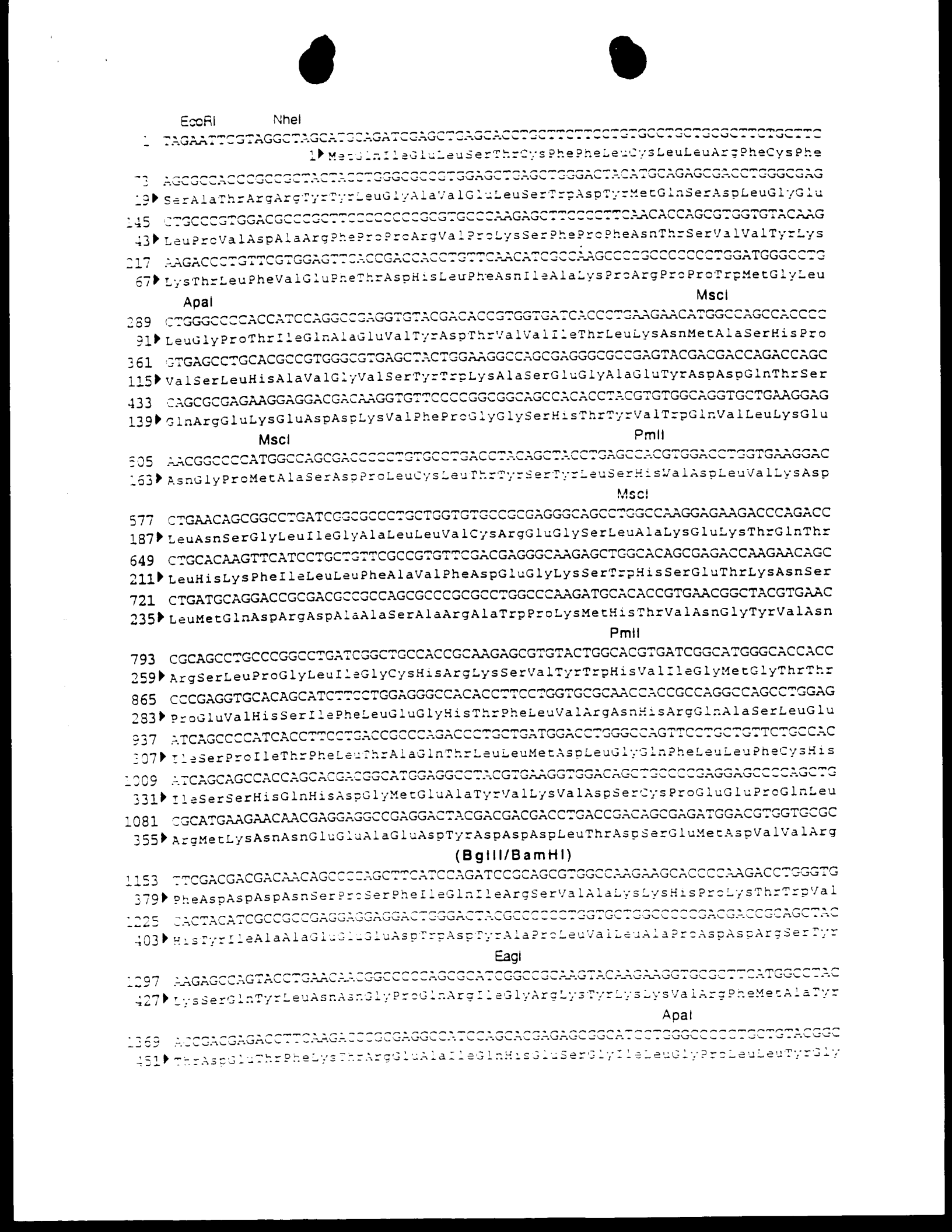
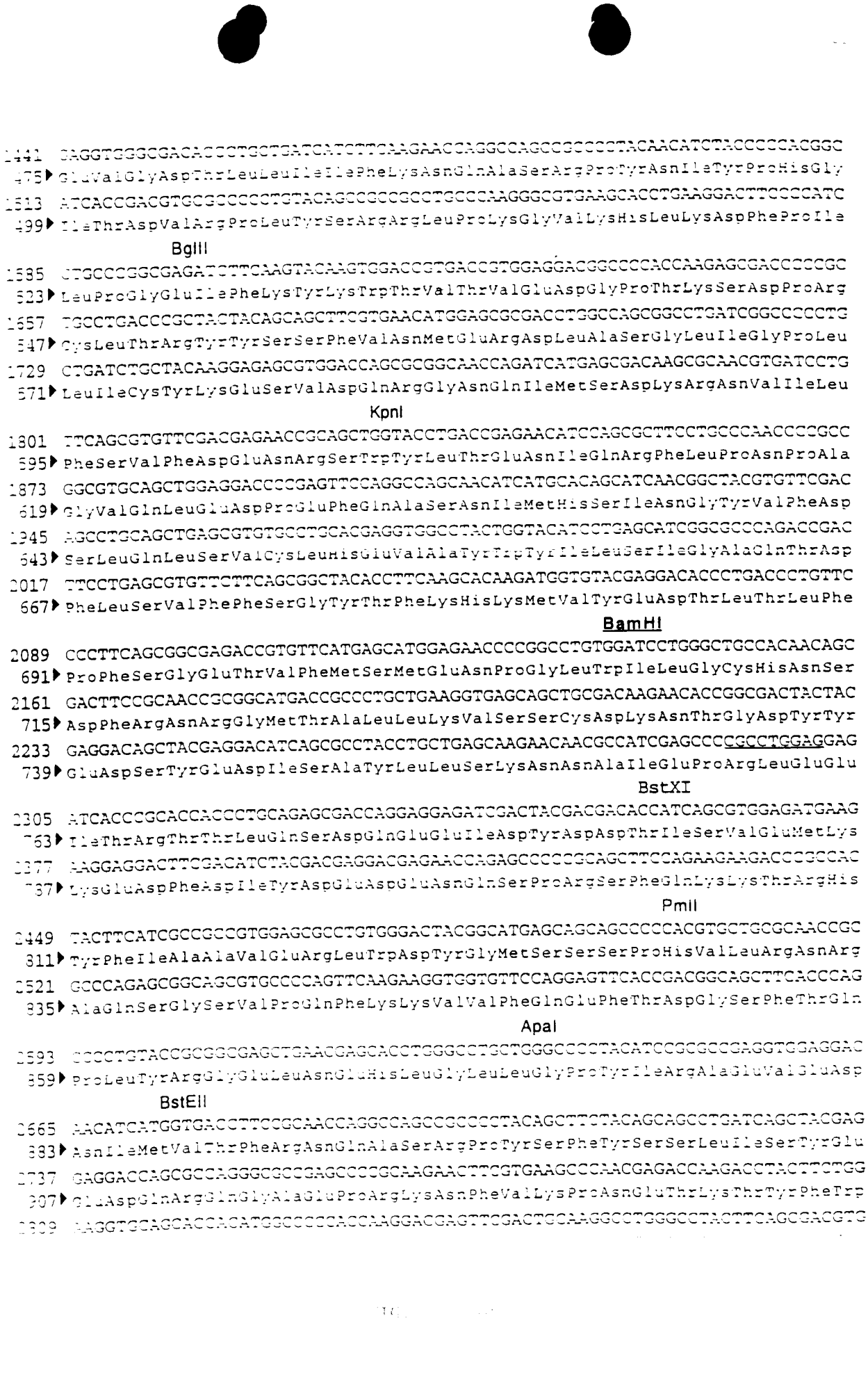
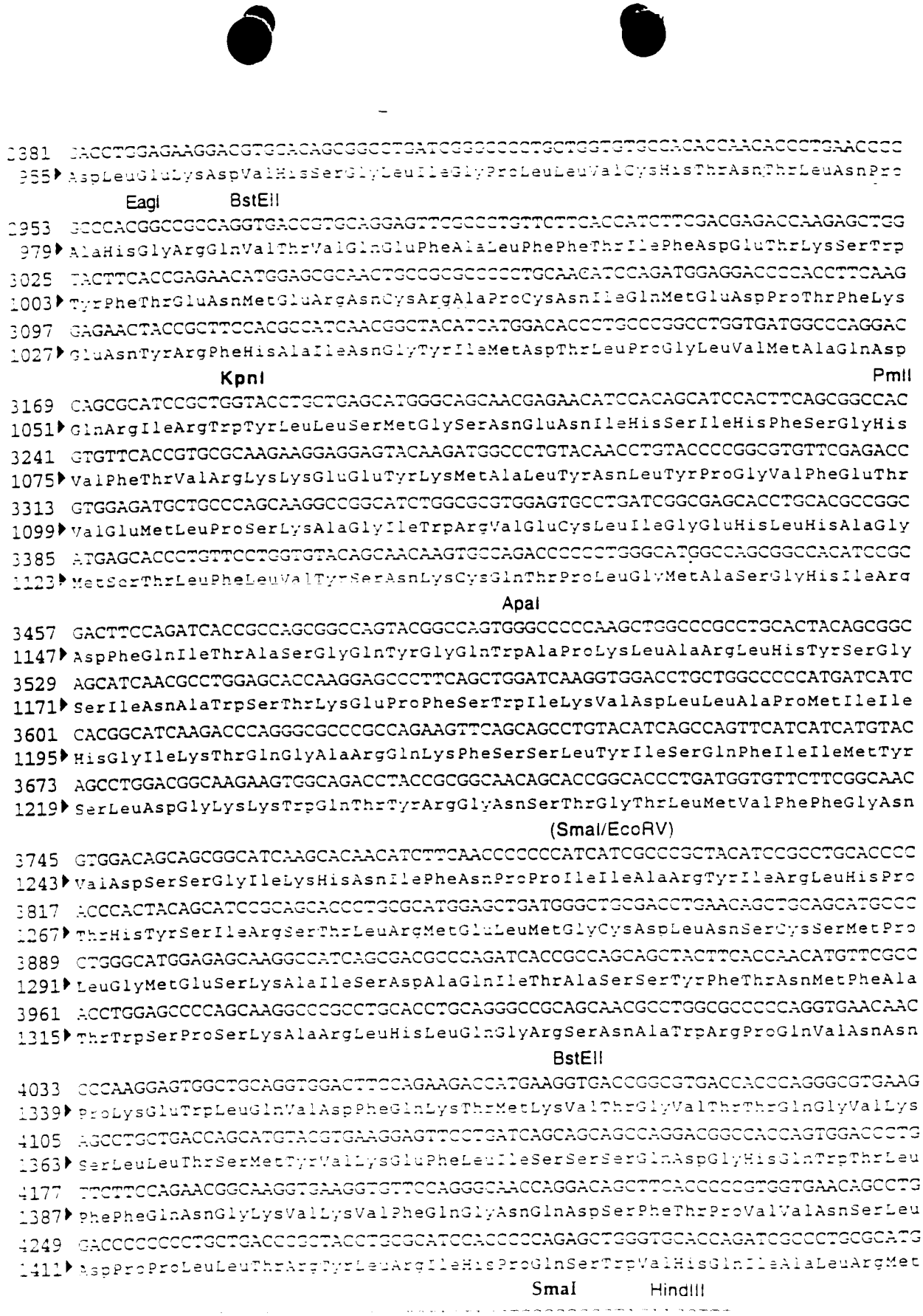

EcoRI
ApaI
PmlI
PmlI
BamHI
BglII
NheI
ABCD
HindIII
EcoRI
BamHI
ApaI
BglII
KpnI
BamHI
EFG
Insert BamHI into BglII-BamHI
cloning, map with BglII & HindIII
EcoRI
ApaI
PmlI
PmlI
BglII/BamHI
ApaI
KpnI
HindIII
BamHI
NheI
ABCDEFG
BglII
EcoRI
BamHI
PmlI
ApaI
BstEII
BstEII
KpnI
HindIII
HIJ
EcoRI
KpnI
PmlI
ApaI
(SmaI/EcoRV)
BstEII
HindIII
KLMN
insert KpnI-HindIII
EcoRI
BamHI
PmlI
ApaI
BstEII
BstEII
KpnI
PmlI
ApaI
(SmaI/EcoRV)
BstEII
HindIII
SmaI
HIJKLMN
Insert BamHI-HindIII
EcoRI
NheI
ApaI
PmlI
PmlI
(BglII/BamHI)
ApaI
BglII
KpnI
BamHI
PmlI
ApaI
BstEII
BstEII
KpnI
PmlI
ApaI
(SmaI/EcoRV)
BstEII
HindIII
ABCDEFGHIJKLMN, i.e. pAM1-1

FIG. 6 (5 of 5)


EcoRI
NheI
1 TAGAATTCGTAGGCTAGCATGCAGATCGAGCTGAGCACCTGCTTCTTCCTGTGCCTGCTGCGCTTCTGCTTC
1 MetGlnIleGluLeuSerThrCysPhePheLeuCysLeuLeuArgPheCysPhe
73 AGCGCCACCCGCCGCTACTACCTGGGCGCCGTGGAGCTGAGCTGGGACTACATGCAGAGCGACCTGGGCGAG
19 SerAlaThrArgArgTyrTyrLeuGlyAlaValGluLeuSerTrpAspTyrMetGlnSerAspLeuGlyGlu
145 CTGCCCGTGGACGCCCGCTTCCCCCCCCGCGTGCCCAAGAGCTTCCCCTTCAACACCAGCGTGGTGTACAAG
43 LeuProValAspAlaArgPheProProArgValProLysSerPheProPheAsnThrSerValValTyrLys
217 AAGACCCTGTTCGTGGAGTTCACCGACCACCTGTTCAACATCGCCAAGCCCCGCCCCCCCTGGATGGGCCTG
67 LysThrLeuPheValGluPheThrAspHisLeuPheAsnIleAlaLysProArgProProTrpMetGlyLeu
ApaI
MscI
289 CTGGGCCCCACCATCCAGGCCGAGGTGTACGACACCGTGGTGATCACCCTGAAGAACATGGCCAGCCACCCC
91 LeuGlyProThrIleGlnAlaGluValTyrAspThrValValIleThrLeuLysAsnMetAlaSerHisPro
361 GTGAGCCTGCACGCCGTGGGCGTGAGCTACTGGAAGGCCAGCGAGGGCGCCGAGTACGACGACCAGACCAGC
115 ValSerLeuHisAlaValGlyValSerTyrTrpLysAlaSerGluGlyAlaGluTyrAspAspGlnThrSer
433 CAGCGCGAGAAGGAGGACGACAAGGTGTTCCCCGGCGGCAGCCACACCTACGTGTGGCAGGTGCTGAAGGAG
139 GlnArgGluLysGluAspAspLysValPheProGlyGlySerHisThrTyrValTrpGlnValLeuLysGlu
MscI
PmlI
505 AACGGCCCCATGGCCAGCGACCCCCTGTGCCTGACCTACAGCTACCTGAGCCACGTGGACCTGGTGAAGGAC
163 AsnGlyProMetAlaSerAspProLeuCysLeuThrTyrSerTyrLeuSerHisValAspLeuValLysAsp
MscI
577 CTGAACAGCGGCCTGATCGGCGCCCTGCTGGTGTGCCGCGAGGGCAGCCTGGCCAAGGAGAAGACCCAGACC
187 LeuAsnSerGlyLeuIleGlyAlaLeuLeuValCysArgGluGlySerLeuAlaLysGluLysThrGlnThr
649 CTGCACAAGTTCATCCTGCTGTTCGCCGTGTTCGACGAGGGCAAGAGCTGGCACAGCGAGACCAAGAACAGC
211 LeuHisLysPheIleLeuLeuPheAlaValPheAspGluGlyLysSerTrpHisSerGluThrLysAsnSer
721 CTGATGCAGGACCGCGACGCCGCCAGCGCCCGCGCCTGGCCCAAGATGCACACCGTGAACGGCTACGTGAAC
235 LeuMetGlnAspArgAspAlaAlaSerAlaArgAlaTrpProLysMetHisThrValAsnGlyTyrValAsn
PmlI
793 CGCAGCCTGCCCGGCCTGATCGGCTGCCACCGCAAGAGCGTGTACTGGCACGTGATCGGCATGGGCACCACC
259 ArgSerLeuProGlyLeuIleGlyCysHisArgLysSerValTyrTrpHisValIleGlyMetGlyThrThr
865 CCCGAGGTGCACAGCATCTTCCTGGAGGGCCACACCTTCCTGGTGCGCAACCACCGCCAGGCCAGCCTGGAG
283 ProGluValHisSerIlePheLeuGluGlyHisThrPheLeuValArgAsnHisArgGlnAlaSerLeuGlu
937 ATCAGCCCCATCACCTTCCTGACCGCCCAGACCCTGCTGATGGACCTGGGCCAGTTCCTGCTGTTCTGCCAC
307 IleSerProIleThrPheLeuThrAlaGlnThrLeuLeuMetAspLeuGlyGlnPheLeuLeuPheCysHis
1009 ATCAGCAGCCACCAGCACGACGGCATGGAGGCCTACGTGAAGGTGGACAGCTGCCCCGAGGAGCCCCAGCTG
331 IleSerSerHisGlnHisAspGlyMetGluAlaTyrValLysValAspSerCysProGluGluProGlnLeu
1081 CGCATGAAGAACAACGAGGAGGCCGAGGACTACGACGACGACCTGACCGACAGCGAGATGGACGTGGTGCGC
355 ArgMetLysAsnAsnGluGluAlaGluAspTyrAspAspAspLeuThrAspSerGluMetAspValValArg
(BglII/BamHI)
1153 TTCGACGACGACAACAGCCCCAGCTTCATCCAGATCCGCAGCGTGGCCAAGAAGCACCCCAAGACCTGGGTG
379 PheAspAspAspAsnSerProSerPheIleGlnIleArgSerValAlaLysLysHisProLysThrTrpVal
1225 CACTACATCGCCGCCGAGGAGGAGGACTGGGACTACGCCCCCCTGGTGCTGGCCCCCGACGACCGCAGCTAC
403 HisTyrIleAlaAlaGluGluGluAspTrpAspTyrAlaProLeuValLeuAlaProAspAspArgSerTyr
EagI
1297 AAGAGCCAGTACCTGAACAACGGCCCCCAGCGCATCGGCCGCAAGTACAAGAAGGTGCGCTTCATGGCCTAC
427 LysSerGlnTyrLeuAsnAsnGlyProGlnArgIleGlyArgLysTyrLysLysValArgPheMetAlaTyr
ApaI
1369 ACCGACGAGACCTTCAAGACCCGCGAGGCCATCCAGCACGAGAGCGGCATCCTGGGCCCCCTGCTGTACGGC
451 ThrAspGluThrPheLysThrArgGluAlaIleGlnHisGluSerGlyIleLeuGlyProLeuLeuTyrGly


1441 GAGGTGGGCGACACCCTGCTGATCATCTTCAAGAACCAGGCCAGCCCCCCTACAACATCTACCCCCACGGC
475 GluValGlyAspThrLeuLeuIleIlePheLysAsnGlnAlaSerArgProTyrAsnIleTyrProHisGly
1513 ATCACCGACGTGCGCCCCCTGTACAGCCGCCGCCTGCCCAAGGGCGTGAAGCACCTGAAGGACTTCCCCATC
499 IleThrAspValArgProLeuTyrSerArgArgLeuProLysGlyValLysHisLeuLysAspPheProIle
BglII
1585 CTGCCCGGCGAGATCTTCAAGTACAAGTGGACCGTGACCGTGGAGGACGGCCCCACCAAGAGCGACCCCCGC
523 LeuProGlyGluIlePheLysTyrLysTrpThrValThrValGluAspGlyProThrLysSerAspProArg
1657 TGCCTGACCCGCTACTACAGCAGCTTCGTGAACATGGAGCGCGACCTGGCCAGCGGCCTGATCGGCCCCCTG
547 CysLeuThrArgTyrTyrSerSerPheValAsnMetGluArgAspLeuAlaSerGlyLeuIleGlyProLeu
1729 CTGATCTGCTACAAGGAGAGCGTGGACCAGCGCGGCAACCAGATCATGAGCGACAAGCGCAACGTGATCCTG
571 LeuIleCysTyrLysGluSerValAspGlnArgGlyAsnGlnIleMetSerAspLysArgAsnValIleLeu
KpnI
1801 TTCAGCGTGTTCGACGAGAACCGCAGCTGGTACCTGACCGAGAACATCCAGCGCTTCCTGCCCAACCCCGCC
595 PheSerValPheAspGluAsnArgSerTrpTyrLeuThrGluAsnIleGlnArgPheLeuProAsnProAla
1873 GGCGTGCAGCTGGAGGACCCCGAGTTCCAGGCCAGCAACATCATGCACAGCATCAACGGCTACGTGTTCGAC
619 GlyValGlnLeuGluAspProGluPheGlnAlaSerAsnIleMetHisSerIleAsnGlyTyrValPheAsp
1945 AGCCTGCAGCTGAGCGTGTGCCTGCACGAGGTGGCCTACTGGTACATCCTGAGCATCGGCGCCCAGACCGAC
643 SerLeuGlnLeuSerValCysLeuHisGluValAlaTyrTrpTyrIleLeuSerIleGlyAlaGlnThrAsp
2017 TTCCTGAGCGTGTTCTTCAGCGGCTACACCTTCAAGCACAAGATGGTGTACGAGGACACCCTGACCCTGTTC
667 PheLeuSerValPhePheSerGlyTyrThrPheLysHisLysMetValTyrGluAspThrLeuThrLeuPhe
BamHI
2089 CCCTTCAGCGGCGAGACCGTGTTCATGAGCATGGAGAACCCCGGCCTGTGGATCCTGGGCTGCCACAACAGC
691 ProPheSerGlyGluThrValPheMetSerMetGluAsnProGlyLeuTrpIleLeuGlyCysHisAsnSer
2161 GACTTCCGCAACCGCGGCATGACCGCCCTGCTGAAGGTGAGCAGCTGCGACAAGAACACCGGCGACTACTAC
715 AspPheArgAsnArgGlyMetThrAlaLeuLeuLysValSerSerCysAspLysAsnThrGlyAspTyrTyr
2233 GAGGACAGCTACGAGGACATCAGCGCCTACCTGCTGAGCAAGAACAACGCCATCGAGCCCCGCCTGGAGGAG
739 GluAspSerTyrGluAspIleSerAlaTyrLeuLeuSerLysAsnAsnAlaIleGluProArgLeuGluGlu
BstXI
2305 ATCACCCGCACCACCCTGCAGAGCGACCAGGAGGAGATCGACTACGACGACACCATCAGCGTGGAGATGAAG
763 IleThrArgThrThrLeuGlnSerAspGlnGluGluIleAspTyrAspAspThrIleSerValGluMetLys
2377 AAGGAGGACTTCGACATCTACGACGAGGACGAGAACCAGAGCCCCCGCAGCTTCCAGAAGAAGACCCGCCAC
787 LysGluAspPheAspIleTyrAspGluAspGluAsnGlnSerProArgSerPheGlnLysLysThrArgHis
PmlI
2449 TACTTCATCGCCGCCGTGGAGCGCCTGTGGGACTACGGCATGAGCAGCAGCCCCCACGTGCTGCGCAACCGC
811 TyrPheIleAlaAlaValGluArgLeuTrpAspTyrGlyMetSerSerSerProHisValLeuArgAsnArg
2521 GCCCAGAGCGGCAGCGTGCCCCAGTTCAAGAAGGTGGTGTTCCAGGAGTTCACCGACGGCAGCTTCACCCAG
835 AlaGlnSerGlySerValProGlnPheLysLysValValPheGlnGluPheThrAspGlySerPheThrGln
ApaI
2593 CCCCTGTACCGCGGCGAGCTGAACGAGCACCTGGGCCTGCTGGGCCCCTACATCCGCGCCGAGGTGGAGGAC
859 ProLeuTyrArgGlyGluLeuAsnGluHisLeuGlyLeuLeuGlyProTyrIleArgAlaGluValGluAsp
BstEII
2665 AACATCATGGTGACCTTCCGCAACCAGGCCAGCCGCCCCTACAGCTTCTACAGCAGCCTGATCAGCTACGAG
883 AsnIleMetValThrPheArgAsnGlnAlaSerArgProTyrSerPheTyrSerSerLeuIleSerTyrGlu
2737 GAGGACCAGCGCCAGGGCGCCGAGCCCCGCAAGAACTTCGTGAAGCCCAACGAGACCAAGACCTACTTCTGG
907 GluAspGlnArgGlnGlyAlaGluProArgLysAsnPheValLysProAsnGluThrLysThrTyrPheTrp
2809 AAGGTGCAGCACCACATGGCCCCCACCAAGGACGAGTTCGACTGCAAGGCCTGGGCCTACTTCAGCGACGTG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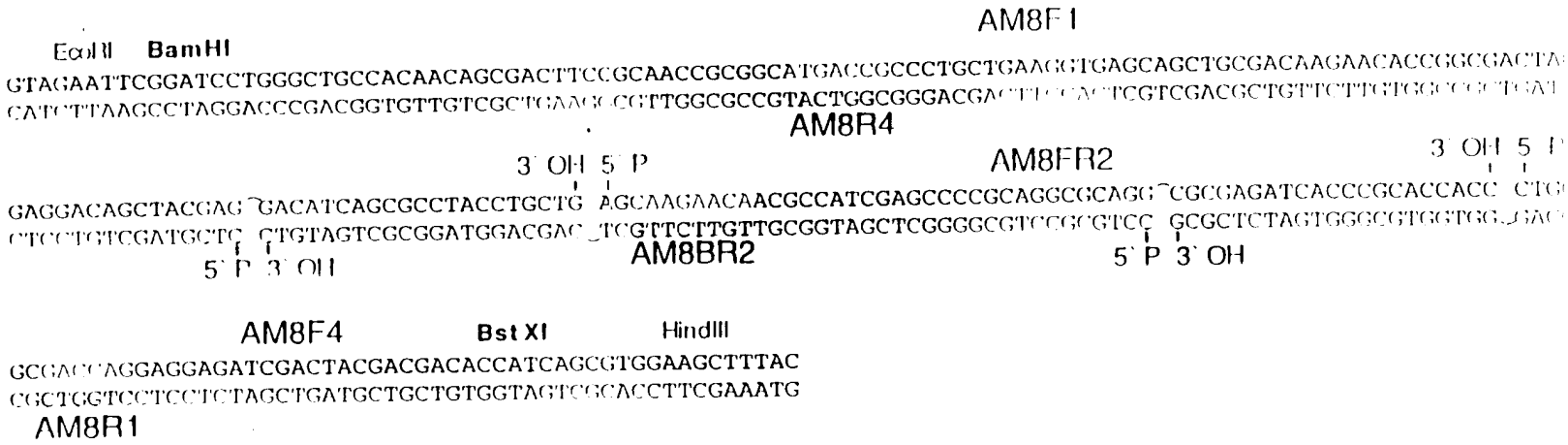
955 AspLeuGluLysAspValHisSerGlyLeuIleGlyProLeuLeuValCysHisThrAsnThrLeuAsnPro
EagI BstEII
2953 GCCCACGGCCGCCAGGTGACCGTGCAGGAGTTCGCCCTGTTCTTCACCATCTTCGACGAGACCAAGAGCTGG
979 AlaHisGlyArgGlnValThrValGlnGluPheAlaLeuPhePheThrIlePheAspGluThrLysSerTrp
3025 TACTTCACCGAGAACATGGAGCGCAACTGCCGCGCCCCCTGCAACATCCAGATGGAGGACCCCACCTTCAAG
1003 TyrPheThrGluAsnMetGluArgAsnCysArgAlaProCysAsnIleGlnMetGluAspProThrPheLys
3097 GAGAACTACCGCTTCCACGCCATCAACGGCTACATCATGGACACCCTGCCCGGCCTGGTGATGGCCCAGGAC
1027 GluAsnTyrArgPheHisAlaIleAsnGlyTyrIleMetAspThrLeuProGlyLeuValMetAlaGlnAsp
KpnI PmlI
3169 CAGCGCATCCGCTGGTACCTGCTGAGCATGGGCAGCAACGAGAACATCCACAGCATCCACTTCAGCGGCCAC
1051 GlnArgIleArgTrpTyrLeuLeuSerMetGlySerAsnGluAsnIleHisSerIleHisPheSerGlyHis
3241 GTGTTCACCGTGCGCAAGAAGGAGGAGTACAAGATGGCCCTGTACAACCTGTACCCCGGCGTGTTCGAGACC
1075 ValPheThrValArgLysLysGluGluTyrLysMetAlaLeuTyrAsnLeuTyrProGlyValPheGluThr
3313 GTGGAGATGCTGCCCAGCAAGGCCGGCATCTGGCGCGTGGAGTGCCTGATCGGCGAGCACCTGCACGCCGGC
1099 ValGluMetLeuProSerLysAlaGlyIleTrpArgValGluCysLeuIleGlyGluHisLeuHisAlaGly
3385 ATGAGCACCCTGTTCCTGGTGTACAGCAACAAGTGCCAGACCCCCCTGGGCATGGCCAGCGGCCACATCCGC
1123 MetSerThrLeuPheLeuValTyrSerAsnLysCysGlnThrProLeuGlyMetAlaSerGlyHisIleArg
ApaI
3457 GACTTCCAGATCACCGCCAGCGGCCAGTACGGCCAGTGGGCCCCCAAGCTGGCCCGCCTGCACTACAGCGGC
1147 AspPheGlnIleThrAlaSerGlyGlnTyrGlyGlnTrpAlaProLysLeuAlaArgLeuHisTyrSerGly
3529 AGCATCAACGCCTGGAGCACCAAGGAGCCCTTCAGCTGGATCAAGGTGGACCTGCTGGCCCCCATGATCATC
1171 SerIleAsnAlaTrpSerThrLysGluProPheSerTrpIleLysValAspLeuLeuAlaProMetIleIle
3601 CACGGCATCAAGACCCAGGGCGCCCGCCAGAAGTTCAGCAGCCTGTACATCAGCCAGTTCATCATCATGTAC
1195 HisGlyIleLysThrGlnGlyAlaArgGlnLysPheSerSerLeuTyrIleSerGlnPheIleIleMetTyr
3673 AGCCTGGACGGCAAGAAGTGGCAGACCTACCGCGGCAACAGCACCGGCACCCTGATGGTGTTCTTCGGCAAC
1219 SerLeuAspGlyLysLysTrpGlnThrTyrArgGlyAsnSerThrGlyThrLeuMetValPhePheGlyAsn
(SmaI/EcoRV)
3745 GTGGACAGCAGCGGCATCAAGCACAACATCTTCAACCCCCCCATCATCGCCCGCTACATCCGCCTGCACCCC
1243 ValAspSerSerGlyIleLysHisAsnIlePheAsnProProIleIleAlaArgTyrIleArgLeuHisPro
3817 ACCCACTACAGCATCCGCAGCACCCTGCGCATGGAGCTGATGGGCTGCGACCTGAACAGCTGCAGCATGCCC
1267 ThrHisTyrSerIleArgSerThrLeuArgMetGluLeuMetGlyCysAspLeuAsnSerCysSerMetPro
3889 CTGGGCATGGAGAGCAAGGCCATCAGCGACGCCCAGATCACCGCCAGCAGCTACTTCACCAACATGTTCGCC
1291 LeuGlyMetGluSerLysAlaIleSerAspAlaGlnIleThrAlaSerSerTyrPheThrAsnMetPheAla
3961 ACCTGGAGCCCCAGCAAGGCCCGCCTGCACCTGCAGGGCCGCAGCAACGCCTGGCGCCCCCAGGTGAACAAC
1315 ThrTrpSerProSerLysAlaArgLeuHisLeuGlnGlyArgSerAsnAlaTrpArgProGlnValAsnAsn
BstEII
4033 CCCAAGGAGTGGCTGCAGGTGGACTTCCAGAAGACCATGAAGGTGACCGGCGTGACCACCCAGGGCGTGAAG
1339 ProLysGluTrpLeuGlnValAspPheGlnLysThrMetLysValThrGlyValThrThrGlnGlyValLys
4105 AGCCTGCTGACCAGCATGTACGTGAAGGAGTTCCTGATCAGCAGCAGCCAGGACGGCCACCAGTGGACCCTG
1363 SerLeuLeuThrSerMetTyrValLysGluPheLeuIleSerSerSerGlnAspGlyHisGlnTrpThrLeu
4177 TTCTTCCAGAACGGCAAGGTGAAGGTGTTCCAGGGCAACCAGGACAGCTTCACCCCCGTGGTGAACAGCCTG
1387 PhePheGlnAsnGlyLysValLysValPheGlnGlyAsnGlnAspSerPheThrProValValAsnSerLeu
4249 GACCCCCCCCTGCTGACCCGCTACCTGCGCATCCACCCCCAGAGCTGGGTGCACCAGATCGCCCTGCGCATG
1411 AspProProLeuLeuThrArgTyrLeuArgIleHisProGlnSerTrpValHisGlnIleAlaLeuArgMet
SmaI HindIII

FIG. 7 (3 of 3)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EcoRI
BamHI
AM8R4
3` OH 5` P
AM8FR2
3` OH 5` P
5` P 3` OH
AM8BR2
5` P 3` OH
AM8F4
Bst XI
HindIII
AM8R1

FIG. 8

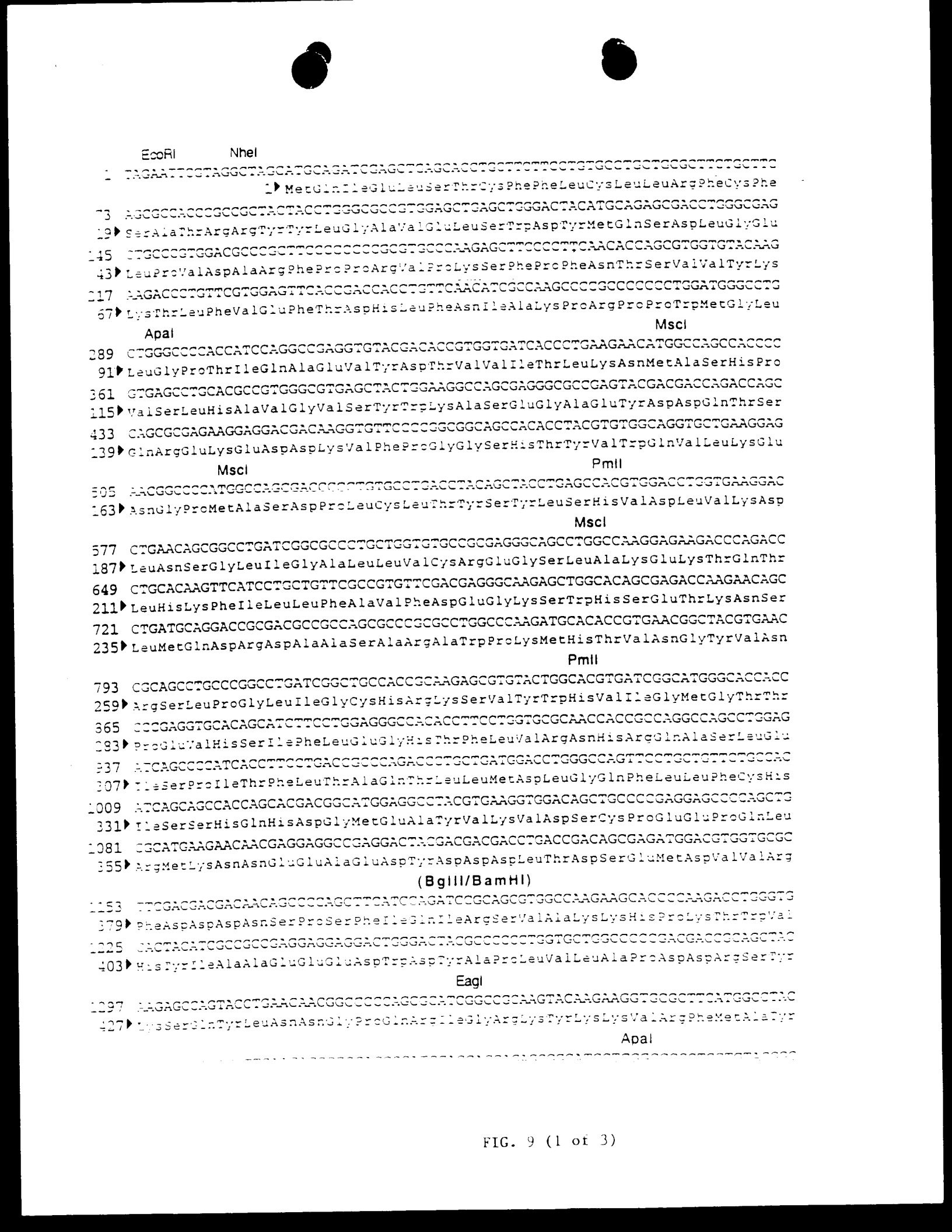

EcoRI
NheI
MscI
ApaI
MscI
PmlI
MscI
PmlI
(BglII/BamHI)
EagI
ApaI

FIG. 9 (1 of 3)

1441 GAGGTGGGCGACACCCTGCTGATCATCTTCAAGAACCAGGCCAGCCCCCCTACAACATCTACCCCCACGGC
475 GluValGlyAspThrLeuLeuIlePheLysAsnGlnAlaSerArgProTyrAsnIleTyrProHisGly
1513 ATCACCGACGTGCGCCCCCTGTACAGCCGCCGCCTGCCCAAGGGCGTGAAGCACCTGAAGGACTTCCCCATC
499 IleThrAspValArgProLeuTyrSerArgArgLeuProLysGlyValLysHisLeuLysAspPheProIle
BglII
1585 CTGCCCGGCGAGATCTTCAAGTACAAGTGGACCGTGACCGTGGAGGACGGCCCCACCAAGAGCGACCCCCGC
523 LeuProGlyGluIlePheLysTyrLysTrpThrValThrValGluAspGlyProThrLysSerAspProArg
1657 TGCCTGACCCGCTACTACAGCAGCTTCGTGAACATGGAGCGCGACCTGGCCAGCGGCCTGATCGGCCCCCTG
547 CysLeuThrArgTyrTyrSerSerPheValAsnMetGluArgAspLeuAlaSerGlyLeuIleGlyProLeu
1729 CTGATCTGCTACAAGGAGAGCGTGGACCAGCGCGGCAACCAGATCATGAGCGACAAGCGCAACGTGATCCTG
571 LeuIleCysTyrLysGluSerValAspGlnArgGlyAsnGlnIleMetSerAspLysArgAsnValIleLeu
KpnI
1801 TTCAGCGTGTTCGACGAGAACCGCAGCTGGTACCTGACCGAGAACATCCAGCGCTTCCTGCCCAACCCCGCC
595 PheSerValPheAspGluAsnArgSerTrpTyrLeuThrGluAsnIleGlnArgPheLeuProAsnProAla
1873 GGCGTGCAGCTGGAGGACCCCGAGTTCCAGGCCAGCAACATCATGCACAGCATCAACGGCTACGTGTTCGAC
619 GlyValGlnLeuGluAspProGluPheGlnAlaSerAsnIleMetHisSerIleAsnGlyTyrValPheAsp
1945 AGCCTCCAGCTGAGCGTGTGCCTGCACGAGGTGGCCTACTGGTACATCCTGAGCATCGGCGCCCAGACCGAC
643 SerLeuGlnLeuSerValCysLeuHisGluValAlaTyrTrpTyrIleLeuSerIleGlyAlaGlnThrAsp
2017 TTCCTGAGCGTGTTCTTCAGCGGCTACACCTTCAAGCACAAGATGGTGTACGAGGACACCCTGACCCTGTTC
667 PheLeuSerValPhePheSerGlyTyrThrPheLysHisLysMetValTyrGluAspThrLeuThrLeuPhe
BamHI
2089 CCCTTCAGCGGCGAGACCGTGTTCATGAGCATGGAGAACCCCGGCCTGTGGATCCTGGGCTGCCACAACAGC
691 ProPheSerGlyGluThrValPheMetSerMetGluAsnProGlyLeuTrpIleLeuGlyCysHisAsnSer
2161 GACTTCCGCAACCGCGGCATGACCGCCCTGCTGAAGGTGAGCAGCTGCGACAAGAACACCGGCGACTACTAC
715 AspPheArgAsnArgGlyMetThrAlaLeuLeuLysValSerSerCysAspLysAsnThrGlyAspTyrTyr
2233 GAGGACAGCTACGAGGACATCAGCGCCTACCTGCTGAGCAAGAACAACGCCATCGAGCCCCGCAGGCGCAGG
739 GluAspSerTyrGluAspIleSerAlaTyrLeuLeuSerLysAsnAsnAlaIleGluProArgArgArgArg
BstXI
2305 CGCGAGATCACCCGCACCACCCTGCAGAGCGACCAGGAGGAGATCGACTACGACGACACCATCAGCGTGGAG
763 ArgGluIleThrArgThrThrLeuGlnSerAspGlnGluGluIleAspTyrAspAspThrIleSerValGlu
2377 ATGAAGAAGGAGGACTTCGACATCTACGACGAGGACGAGAACCAGAGCCCCCGCAGCTTCCAGAAGAAGACC
787 MetLysLysGluAspPheAspIleTyrAspGluAspGluAsnGlnSerProArgSerPheGlnLysLysThr
PmlI
2449 CGCCACTACTTCATCGCCGCCGTGGAGCGCCTGTGGGACTACGGCATGAGCAGCAGCCCCCACGTGCTGCGC
811 ArgHisTyrPheIleAlaAlaValGluArgLeuTrpAspTyrGlyMetSerSerSerProHisValLeuArg
2521 AACCGCGCCCAGAGCGGCAGCGTGCCCCAGTTCAAGAAGGTGGTGTTCCAGGAGTTCACCGACGGCAGCTTC
835 AsnArgAlaGlnSerGlySerValProGlnPheLysLysValValPheGlnGluPheThrAspGlySerPhe
ApaI
2593 AGCCAGCCCCTGTACCGCGGCGAGCTGAACGAGCACCTGGGCCTGCTGGGCCCCTACATCCGCGCCGAGGTG
859 ThrGlnProLeuTyrArgGlyGluLeuAsnGluHisLeuGlyLeuLeuGlyProTyrIleArgAlaGluVal
BstEII
2665 GAGGACAACATCATGGTGACCTTCCGCAACCAGGCCAGCCGCCCCTACAGCTTCTACAGCAGCCTGATCAGC
883 GluAspAsnIleMetValThrPheArgAsnGlnAlaSerArgProTyrSerPheTyrSerSerLeuIleSer
2737 TACGAGGAGGACCAGCGCCAGGGCGCCGAGCCCCGCAAGAACTTCGTGAAGCCCAACGAGACCAAGACCTAC
907 TyrGluGluAspGlnArgGlnGlyAlaGluProArgLysAsnPheValLysProAsnGluThrLysThrTyr

FIG. 3 (2 of 3)

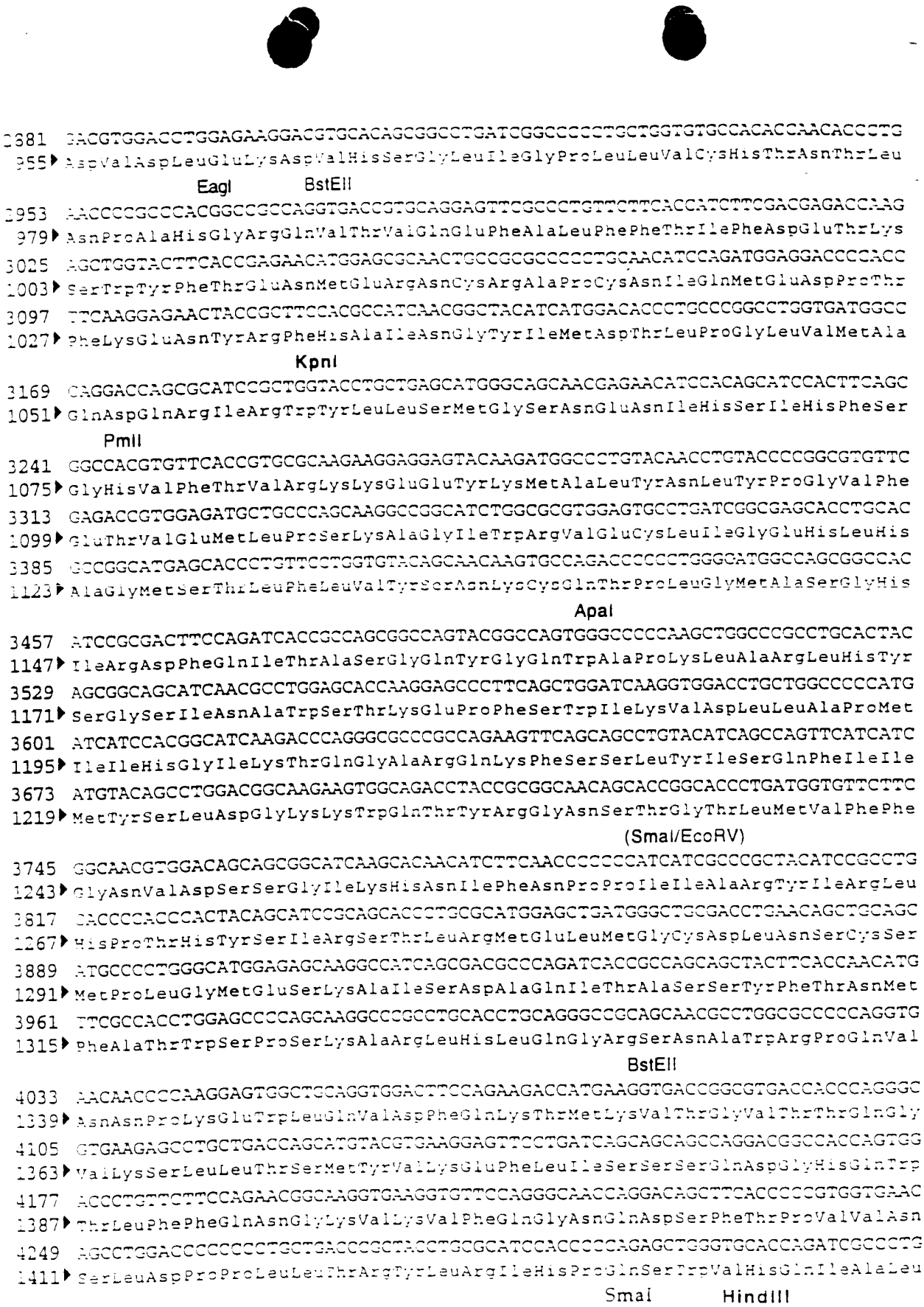
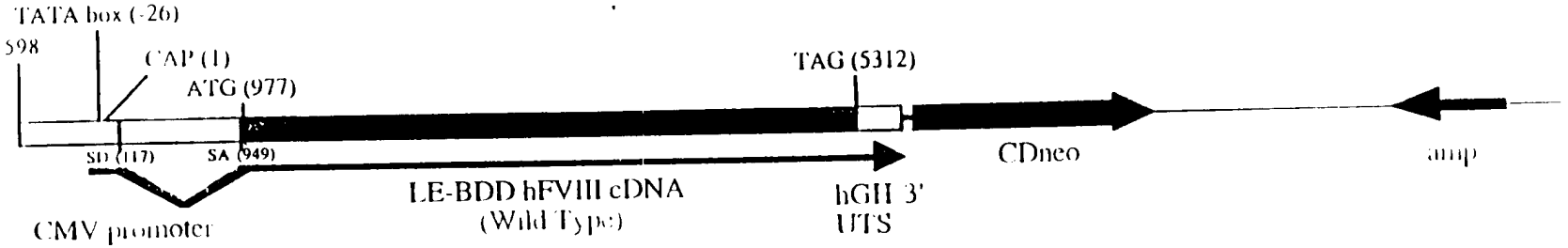

2881 GACGTGGACCTGGAGAAGGACGTGCACAGCGGCCTGATCGGCCCCCTGCTGGTGTGCCACACCAACACCCTG
955 AspValAspLeuGluLysAspValHisSerGlyLeuIleGlyProLeuLeuValCysHisThrAsnThrLeu
EagI
BstEII
2953 AACCCCGCCCACGGCCGCCAGGTGACCGTGCAGGAGTTCGCCCTGTTCTTCACCATCTTCGACGAGACCAAG
979 AsnProAlaHisGlyArgGlnValThrValGlnGluPheAlaLeuPhePheThrIlePheAspGluThrLys
3025 AGCTGGTACTTCACCGAGAACATGGAGCGCAACTGCCGCGCCCCCTGCAACATCCAGATGGAGGACCCCACC
1003 SerTrpTyrPheThrGluAsnMetGluArgAsnCysArgAlaProCysAsnIleGlnMetGluAspProThr
3097 TTCAAGGAGAACTACCGCTTCCACGCCATCAACGGCTACATCATGGACACCCTGCCCGGCCTGGTGATGGCC
1027 PheLysGluAsnTyrArgPheHisAlaIleAsnGlyTyrIleMetAspThrLeuProGlyLeuValMetAla
KpnI
3169 CAGGACCAGCGCATCCGCTGGTACCTGCTGAGCATGGGCAGCAACGAGAACATCCACAGCATCCACTTCAGC
1051 GlnAspGlnArgIleArgTrpTyrLeuLeuSerMetGlySerAsnGluAsnIleHisSerIleHisPheSer
PmlI
3241 GGCCACGTGTTCACCGTGCGCAAGAAGGAGGAGTACAAGATGGCCCTGTACAACCTGTACCCCGGCGTGTTC
1075 GlyHisValPheThrValArgLysLysGluGluTyrLysMetAlaLeuTyrAsnLeuTyrProGlyValPhe
3313 GAGACCGTGGAGATGCTGCCCAGCAAGGCCGGCATCTGGCGCGTGGAGTGCCTGATCGGCGAGCACCTGCAC
1099 GluThrValGluMetLeuProSerLysAlaGlyIleTrpArgValGluCysLeuIleGlyGluHisLeuHis
3385 GCCGGCATGAGCACCCTGTTCCTGGTGTACAGCAACAAGTGCCAGACCCCCCTGGGCATGGCCAGCGGCCAC
1123 AlaGlyMetSerThrLeuPheLeuValTyrSerAsnLysCysGlnThrProLeuGlyMetAlaSerGlyHis
ApaI
3457 ATCCGCGACTTCCAGATCACCGCCAGCGGCCAGTACGGCCAGTGGGCCCCCAAGCTGGCCCGCCTGCACTAC
1147 IleArgAspPheGlnIleThrAlaSerGlyGlnTyrGlyGlnTrpAlaProLysLeuAlaArgLeuHisTyr
3529 AGCGGCAGCATCAACGCCTGGAGCACCAAGGAGCCCTTCAGCTGGATCAAGGTGGACCTGCTGGCCCCCATG
1171 SerGlySerIleAsnAlaTrpSerThrLysGluProPheSerTrpIleLysValAspLeuLeuAlaProMet
3601 ATCATCCACGGCATCAAGACCCAGGGCGCCCGCCAGAAGTTCAGCAGCCTGTACATCAGCCAGTTCATCATC
1195 IleIleHisGlyIleLysThrGlnGlyAlaArgGlnLysPheSerSerLeuTyrIleSerGlnPheIleIle
3673 ATGTACAGCCTGGACGGCAAGAAGTGGCAGACCTACCGCGGCAACAGCACCGGCACCCTGATGGTGTTCTTC
1219 MetTyrSerLeuAspGlyLysLysTrpGlnThrTyrArgGlyAsnSerThrGlyThrLeuMetValPhePhe
(SmaI/EcoRV)
3745 GGCAACGTGGACAGCAGCGGCATCAAGCACAACATCTTCAACCCCCCCATCATCGCCCGCTACATCCGCCTG
1243 GlyAsnValAspSerSerGlyIleLysHisAsnIlePheAsnProProIleIleAlaArgTyrIleArgLeu
3817 CACCCCACCCACTACAGCATCCGCAGCACCCTGCGCATGGAGCTGATGGGCTGCGACCTGAACAGCTGCAGC
1267 HisProThrHisTyrSerIleArgSerThrLeuArgMetGluLeuMetGlyCysAspLeuAsnSerCysSer
3889 ATGCCCCTGGGCATGGAGAGCAAGGCCATCAGCGACGCCCAGATCACCGCCAGCAGCTACTTCACCAACATG
1291 MetProLeuGlyMetGluSerLysAlaIleSerAspAlaGlnIleThrAlaSerSerTyrPheThrAsnMet
3961 TTCGCCACCTGGAGCCCCAGCAAGGCCCGCCTGCACCTGCAGGGCCGCAGCAACGCCTGGCGCCCCCAGGTG
1315 PheAlaThrTrpSerProSerLysAlaArgLeuHisLeuGlnGlyArgSerAsnAlaTrpArgProGlnVal
BstEII
4033 AACAACCCCAAGGAGTGGCTGCAGGTGGACTTCCAGAAGACCATGAAGGTGACCGGCGTGACCACCCAGGGC
1339 AsnAsnProLysGluTrpLeuGlnValAspPheGlnLysThrMetLysValThrGlyValThrThrGlnGly
4105 GTGAAGAGCCTGCTGACCAGCATGTACGTGAAGGAGTTCCTGATCAGCAGCAGCCAGGACGGCCACCAGTGG
1363 ValLysSerLeuLeuThrSerMetTyrValLysGluPheLeuIleSerSerSerGlnAspGlyHisGlnTrp
4177 ACCCTGTTCTTCCAGAACGGCAAGGTGAAGGTGTTCCAGGGCAACCAGGACAGCTTCACCCCCGTGGTGAAC
1387 ThrLeuPhePheGlnAsnGlyLysValLysValPheGlnGlyAsnGlnAspSerPheThrProValValAsn
4249 AGCCTGGACCCCCCCCTGCTGACCCGCTACCTGCGCATCCACCCCCAGAGCTGGGTGCACCAGATCGCCCTG
1411 SerLeuAspProProLeuLeuThrArgTyrLeuArgIleHisProGlnSerTrpValHisGlnIleAlaLeu
SmaI
HindIII

FIG. 9 (3 of 3)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598
CAP (1)
ATG (977)
TAG (5312)
SD (117)
SA (949)
CDneo
amp
CMV promoter
LE-BDD hFVIII cDNA
(Wild Type)
hGH 3'
UTS

FIG. 10

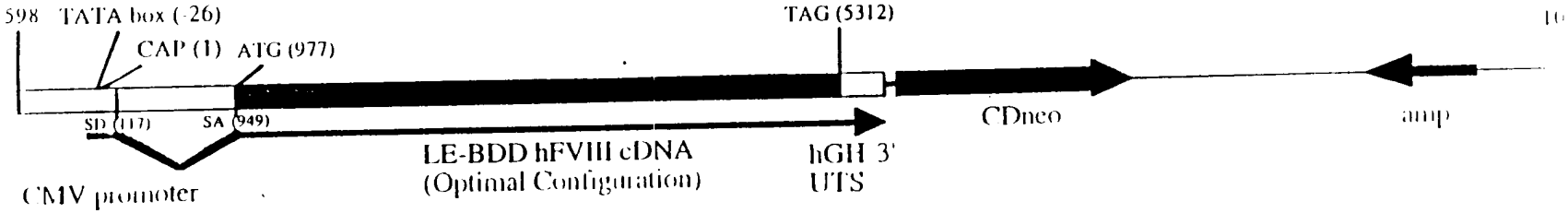
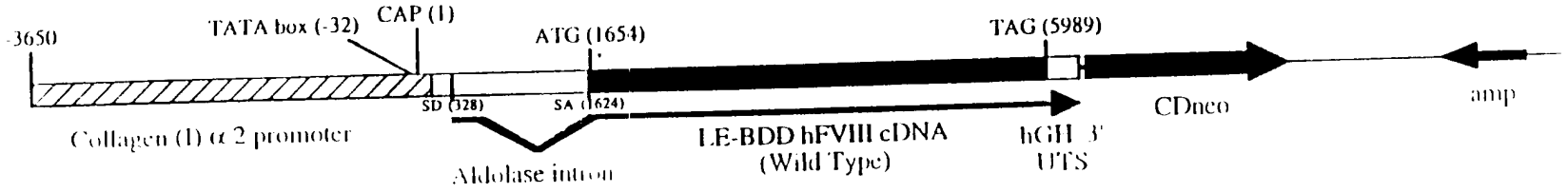

598
TATA box (-26)
CAP (1)
ATG (977)
TAG (5312)
SD (117)
SA (949)
CMV promoter
LE-BDD hFVIII cDNA
(Optimal Configuration)
hGH 3'
UTS
CDneo
amp

FIG. 11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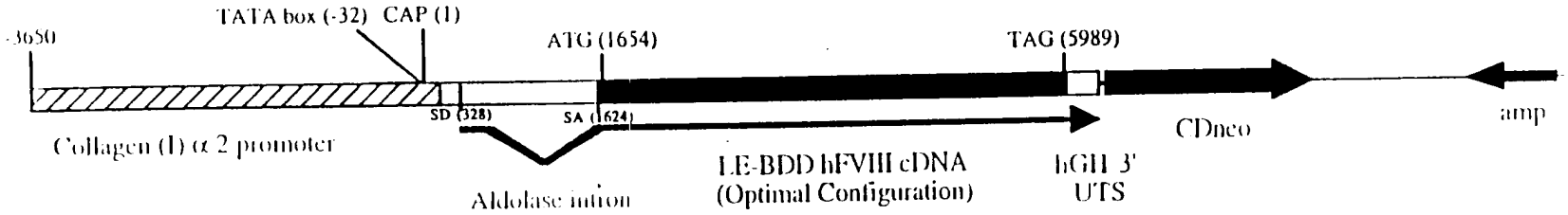
CAP (1)
ATG (1654)
TAG (5989)
SD (328)
SA (1624)
CDneo
amp
Collagen (I) α 2 promoter
Aldolase intron
LE-BDD hFVIII cDNA
(Wild Type)
hGH 3'
UTS

FIG. 12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-3650
ATG (1654)
TAG (5989)
SD (328)
SA (1624)
Collagen (I) α 2 promoter
Aldolase intron
LE-BDD hFVIII cDNA
(Optimal Configuration)
hGH 3'
UTS
CDneo
amp

FIG. 13